To UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: February 7, 2020

SUBJECT: PRIA Draft Risk Assessment for the New Nanosilver Active Ingredient NSPW Nanosilver

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This document provides the human health and ecological risk assessment conducted in support of the proposed registration of Polyguard NSPW Master Batch end use product which contains NSPW Nanosilver as a new antimicrobial pesticide active ingredient (a.i.).

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1 EXECUTIVE SUMMARY

NSPW Nanosilver, which contains nanosilver, was the subject of an application submitted by Nanosilva LLC (hereinafter referred to as Poly-Technical Solutions LTD., due to name change) in August 2009 (MRID 47828900) for use as a material preservative in textiles and plastics. The registrant requested that the nanosilver in NSPW Nanosilver (previously referred to as NSPW-L30SS) be registered as a new active ingredient because it was not an active ingredient in any currently registered pesticide product. NSPW Nanosilver is a liquid suspension containing silica-sulfur-nanosilver particulates where the nanosilver is attached to amorphous silica via a thiolate bond. In 2015, the product was conditionally registered and later challenged in court based on the public interest finding.

The current registration application is for the same active ingredient, formulated as a master batch in an end use product, Polyguard NSPW Master Batch (hereafter referred to as Polyguard). The registrant has also amended the application to include use of NSPW Nanosilver as a material preservation in textiles only. EPA has conducted the following risk assessment based on the assessment prepared to support the 2015 registration, taking into consideration the amended application to limit the use patterns, the most up-to-date science, and the newly submitted ecotoxicity and product chemistry studies.

EPA is making a registration decision for non-food contact uses of NSPW Nanosilver in Polyguard which is incorporated into textiles to suppress the growth of bacteria, algae, fungus, mold, and mildew, which cause odors, discoloration, stains, and deterioration. The finished textiles will contain less than 0.003% silver by weight.

EPA determined that consumers and the environment could be exposed to:

1. Silver ions released from Polyguard;
2. NSPW Nanosilver in Polyguard; and/or
3. Nanosilver particles that might break away from Polyguard

In evaluating the risk from exposure to silver ions, EPA relied on the existing reregistration decision for silver, which concluded that the human health or ecological risk from exposure to silver ions used in water treatment and swimming pools are not of concern (U.S EPA Reregistration Eligibility Decision, 1993). For purposes of evaluating the risk from short-term exposure to NSPW Nanosilver which is contained in Polyguard, the registrant submitted results from acute mammalian-toxicity tests completed using high-level doses of NSPW Nanosilver showing that there were no mortalities or abnormalities in test animals after administration of NSPW Nanosilver by oral, dermal, and inhalation exposure routes. NSPW Nanosilver caused moderate irritation to the eyes of test animals and was not a skin sensitizer. Based on these results, shipping containers filled with NSPW Nanosilver are required to carry a label stating "CAUTION" where contact with eyes or clothing should be avoided. In 2013, EPA waived most

of the required intermediate-term toxicity studies based on low potential exposures to NSPW Nanosilver, and the lack of toxicity noted in the acute animal-toxicity tests completed using high-level doses of NSPW Nanosilver and information from the open literature.

Toxicology Endpoints and Target Margins of Exposure (MOEs)

Although there are no repeat dose toxicity studies available for NSPW Nanosilver, EPA evaluated the risk from occupational and consumer exposure using hazard data available in the scientific literature for other nanosilvers consistent with EPA Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment. The following Points of Departure (PODs)[1] were used for assessing human health risks of the nanosilver in NSPW Nanosilver:

- **Incidental Oral Exposure (Short Term)** – The POD is the No Observed Adverse Effect Level (NOAEL) of 30 mg/kg/day from the 28-day Kim *et al*. (2008) study based on significant increases in serum alkaline phosphatase (ALP) and cholesterol, significant changes in hematology, and accompanied by histopathological evidences of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein) at the Lowest Observed Adverse Effect Level (LOAEL) of 300 mg/kg/day.
- **Incidental Oral Exposure (Intermediate Term)** – The POD is the LOAEL of 30 mg/kg/day from the 90-day Kim *et al*. (2010) study based on histopathological evidences of liver toxicity (bile-duct hyperplasia with focal, multifocal, or lobular necrosis) in both males and females.
- **Dermal Exposure (Short Term)** – The POD is the NOAEL of 30 mg/kg/day from the 28-day Kim *et al.* (2008) study.
- **Dermal Exposure (Intermediate Term)** – The POD is the LOAEL of 30 mg/kg/day from the 90-day Kim *et al.* (2010) study.
- **Dermal Absorption** – The dermal absorption is 6.7 percent based on an observational study by Wan *et al.* (1991), which reported silver concentration in serum as well as that eliminated through urine.

The target MOE is 100 for short-term incidental oral and dermal exposures. This MOE includes uncertainty factors of 10X for interspecies extrapolation and 10X for intraspecies variation. The target MOE is 300 or intermediate-term incidental oral and dermal exposures. This MOE includes the same uncertainty factors of 10X for interspecies extrapolation, 10X for intraspecies variation and an additional 3-fold uncertainty factor to account for the lack of NOAEL.

[1] For the 2015 Registration Decision for NSPW-L30SS, a NOAEL of 49 µg/m^3 from the 12-week Song *et al.* (2012) study was chosen as the POD for inhalation exposure. This POD is not needed for this assessment based on low potential for occupational exposures.

Occupational and Residential Risk Summary

Occupational handler exposures are not expected during the handling of Polyguard, which contains NSPW Nanosilver, because it is formulated as a master batch which is in the form of plastic beads or pellets.

Residential post application dermal and incidental oral exposures are expected because textiles containing NSWP Nanosilver could be used to manufacture apparel and household items such as towels, sheets and blankets. To evaluate dermal and incidental exposure to the nanosilver that might break away from textiles incorporating NSPW Nanosilver, studies were submitted that evaluated the rate of leaching into simulated saliva. These studies did not detect silver in the simulated saliva leachate above the 10 µg silver per liter (µg/L) Limit of Detection (LOD). Consistent with agency practice when no levels are detected, it was assumed that simulated saliva leachate contained a silver concentration of one-half the LOD (*i.e.*, 5 µg/L) and that the silver was in the form of nanosilver as found in NSPW Nanosilver.

The MOEs for incidental oral and dermal exposures were calculated using the results of the simulated saliva leaching study, the POD of 30 mg/kg/day and for dermal exposures, a dermal absorption factor of 6.7 percent, and a body weight of 11 kg for a 1 to <2-year-old child. The incidental oral MOE is 1,200,000 and the dermal MOE is 370,000. These MOEs are well above the target MOEs of 100 and 300 for short- and intermediate-term exposures, respectively, which means that the risks are not of concern.

The MOE for combined exposure was calculated by adding the doses for dermal and incidental oral exposure and using the POD of 30 mg/kg/day, which is applicable to both dermal and incidental oral exposures. The resulting combined MOE is 270,000, which is not of concern because it is greater than the target MOEs of 100 and 300 .

Environmental Risk Summary

Impact to the environment was assessed based on a registrant-submitted daphnid study and estimated environmental exposure from the use of Polyguard, which contains NSPW Nanosilver, in textiles (assuming that 300 million people (U.S. population) each purchased one t-shirt treated with Polyguard). All t-shirts in the textile scenario were assumed to be washed weekly for 52 weeks, releasing 1.6% of the initial silver load as nanosilver per wash to wastewater. The NSPW Nanosilver *Daphnia* study was utilized because the databases and open literature studies for other types of silver (silver ions, nanosilver from other sources, *etc*.) indicate *Daphnia* is the most sensitive species to silver.

The acute risk quotient (RQ) for nanosilver from NSPW Nanosilver for the worst-case, 1Q10 stream flow with low stream dilution was 0.028 and is below the level of concern for listed and non-listed aquatic invertebrates indicating that it is unlikely that the registration of Polyguard as a preservative in textiles will lead to adverse effects for listed or non-listed aquatic organisms.

Nanosilver derived from NSPW Nanosilver as well as silver ions are categorized as very highly toxic to freshwater invertebrates. Although the acute EC_{50} for nanosilver derived from NSPW Nanosilver is below that of silver ions, indicating that it may be more toxic to aquatic species than silver ions from non-nano sources, the endpoints are within the same order of magnitude. No long-term (chronic) risk estimates were evaluated for non-target organisms because no chronic endpoints are available for nanosilver or silver ions derived from NSPW Nanosilver or for parent NSPW Nanosilver. However, risks from chronic exposure are not expected, because the particle is expected to be unstable in water and any chronic exposure would be to silver ions.

Based on the low potential exposure to non-target aquatic and terrestrial organisms, EPA is making a No Effects (NE) determination for all federally-listed threatened/endangered species from the proposed textile use of NSPW Nanosilver as described on the Polyguard label.

2 INTRODUCTION

2.1 Ingredient Profile

Polyguard is formulated using NSPW Nanosilver which is a liquid suspension containing silica-sulfur-nanosilver particulates where the nanosilver is attached to amorphous silica via a thiolate bond. This liquid suspension contains 0.07986 % nanosilver (see Appendix C for calculations and notes on values reported on the label). The particles are formed by reacting silver nitrate with spherical silica particles that have been modified with thiol groups and are suspended in a mixture of water and ethylene glycol. The overall diameter of the particles is 30-50 nm, with silica core particles in the 20-40 nm range and silver particles in the 2-3 nm range (Appendix B and more recently submitted MRID 50649402). The silver particles are bound to the silica core by sulfur.

The current assessment evaluates exposure to NSPW Nanosilver in Polyguard, nanosilver particles that might break away from Polyguard, and silver ions released from Polyguard. In evaluating the risk from exposure to silver ions released from Polyguard, EPA has relied on the existing reregistration decision for silver, which concluded that the human health or ecological risk from exposure to silver ions used in water treatment and swimming pools are not of concern. Silver ions have also been used as a material preservative for coatings and films, textiles and fibers (bedding, apparel, footwear, carpets, draperies, outdoor fabrics, *etc.*), adhesives and sealants, and plastics. These material preservative uses involve no food contact uses, and the products containing the silver ion come in the form of silver ion-polymer complexes, silver ion-

exchange resins, and silver ions embedded in an inert matrix (*e.g.*, glass, zeolite, and apatite). More information on the silver and silver ion case can be found in the 1993 Silver Reregistration Eligibility Document (RED),[2] as well as the registration review documents available at https://www.regulations.gov/ docket ID EPA-HQ-OPP-2009-0334.

2.2 Use Pattern

Polyguard, containing NSPW Nanosilver, is proposed for use in non-food contact textiles such as yarns, filaments, fibers, and knitted, woven, or nonwoven textile fabrics, and subsequent manufactured treated article products. It is intended to suppress the growth of microbes which cause odors, discoloration, stains, and deterioration.

The label states that Polyguard is a polymeric intermediate known as a master batch, which is in the form of plastic beads or pellets. The master batch is then added to the polymer mixture that is used to produce synthetic textile fibers, which are then used to manufacture consumer products. The products include interior use household items such as mops, towels, sheets, mattress covers, draperies, shower curtains, curtains and upholstery, clothing items such as uniforms, socks, tee-shirts, sportswear, and outerwear, and exterior use items such as sail cloth, tents, and awnings.

The maximum application rate for treatment of the finished consumer product is 0.003% (by weight) or 30 ppm of silver. Additional label details are provided in Appendix A.

3 HUMAN HEALTH RISK ASSESSMENT

Nanosilver is a broad-spectrum antimicrobial agent that works by releasing ionic silver, but also exhibits particle-specific effects (Wang *et al*., 2013). In November 2009, EPA convened a meeting of the FIFRA Scientific Advisory Panel (SAP) to address several questions associated with assessing the hazard of and exposure to nanosilver and other nanoscale metal-based pesticides (FIFRA SAP, 2009). In general, the SAP advised that the toxicity of nanosilver could differ from and might be higher than other forms of silver (*e.g*., silver ions). The Panel agreed with EPA that particle size has a substantial impact on particle properties, including rate and concentration of silver ion release, where the effects of size are generally most observable for particles with dimensions below 20 nm and largely below 10 nm (FIFRA SAP 2009, p. 6). In addition to size, other properties such as shape, charge, and surface coating have the potential to impact the biological response to nanosilver.

For NSPW Nanosilver, EPA chose endpoints from the open literature and assessed the risks based on the potential of incidental oral and dermal exposures to treated textiles. The basis for waiving the human health toxicological data which were not required is provided in Appendix E.

[2] Accessed Apr 27, 2019 https://archive.epa.gov/pesticides/reregistration/web/pdf/silver.pdf

For the 2015 Registration Decision for NSPW-L30SS, EPA conducted a search of the open literature on nanosilvers and summarized the results in the decision document for qualitative and quantitative characterization of hazard related to nanosilver particles. The summaries from this search are included as Appendix G of this assessment.

The Agency updated the toxicology database of nanosilvers by performing an open-literature search for recent studies using the US National Library of Medicine (NLM) of the National Institutes of Health (NIH) PubMed[3] on March 14, 2019, to determine if the endpoints previously selected could be updated. The parameters used in the search were (i) nano silver inhalation toxicity, (ii) nanosilver inhalation toxicity, (iii) nano silver dermal toxicity, (iv) nanosilver dermal toxicity, (v) nano silver oral toxicity, and (vi) nanosilver oral toxicity. The search yielded 3 inhalation studies, 1 intratracheal instillation study, 7 oral studies and 3 dermal studies. The studies were reviewed according to the Agency's guidance on reviewing open literature studies. Based on the current selected endpoints and based on the Agency's guidance (U.S. EPA, 2012c), it was determined that none of the studies could be used to revise the current selected points of departures and endpoints. Various reasons for the decision were based on the number of doses tested (not enough doses tested) and the facts that there were no adverse effects observed from exposure, the parameters measured are not used by the Agency to determine adversity, doses were not reported, and/or the adverse effects observed were above the currently selected points of departure or endpoints.

3.1 Summary Toxicity Endpoint and Point of Departure Selections

The toxicological point of departure (POD) is determined from dose-response data and marks the beginning of extrapolation to determine the risk associated with environmentally relevant human exposures. Commonly, this is a NOAEL from a laboratory animal toxicity study, which represents the dose at which no adverse effects were observed in laboratory animals. Oral or dermal subchronic studies are not available for NSPW Nanosilver active ingredient or the nanosilver that might break away from articles incorporating NSPW Nanosilver. In place of these studies, EPA is determining NOAELs and LOAELs from subchronic oral toxicity studies found in the scientific literature for nanosilvers to evaluate the effects that could occur from exposure to the nanosilver present in NSPW Nanosilver.

The SAP cautioned about extrapolating from one nanosilver formulation to another when assessing hazards because differences in particle formulation (*e.g.*, coating and inert ingredients) are likely to affect biological activity, chemical properties and behavior. However, based on the low exposure potential for this product, which is for textiles only and formulated as a master

[3] https://www.ncbi.nlm.nih.gov/pubmed/

batch to limit occupational exposures, and the high MOEs based on PODs chosen in accordance with the open literature[4] guidance, no additional data are needed for this risk assessment.

The oral toxicity studies by Kim *et al*. (2008) and Kim *et al*. (2010) used carboxy-methyl cellulose (CMC)-coated nanosilver with an average diameter of 56 and 60 nm, respectively, which is different from the nanosilver in NSPW Nanosilver which has an overall diameter of 30-50 nm with silica core particles in the 20-40 nm range and silver particles in the 2-3 nm range with no surface coating. These studies were stated as being completed according to OECD guidelines and identified histopathological patterns in the liver that were indicative of distinct adverse effects. EPA has determined that the NOAEL is 30 mg/kg/day from the 28-day Kim *et al*. (2008) study based on significant increases in ALP and cholesterol, significant changes in hematology, and accompanied by histopathological evidences of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein) at the LOAEL dose of 300 mg/kg/day. A NOAEL for the 90-day Kim *et al*. (2010) cannot be established because of adverse histological patterns evident at the lowest dose of 30 mg/kg/day. EPA has determined that the LOAEL from the 90-day Kim *et al*. (2010) study is 30 mg/kg/day based on histopathological evidences of liver toxicity (bile-duct hyperplasia with focal, multifocal, or lobular necrosis) in both males and females.

Based on the above analysis, EPA has determined that the NOAEL of 30 mg/kg/day from the 28-day oral toxicity study by Kim *et al*. (2008) is the POD for short-term oral exposures (<30 days) to the nanosilver in NSPW Nanosilver (Table 1). EPA has also determined that the LOAEL of 30 mg/kg from the 90-day oral toxicity study by Kim *et al*. (2010) is the POD for intermediate-term oral exposures (1 to 6 months) to the nanosilver in NSPW Nanosilver. While the nanosilvers in these studies have larger diameters and different surface coatings from those of the nanosilver in NSPW Nanosilver, they are the closest comparison the Agency has in the available literature. Furthermore, these studies were conducted using OECD guidelines; therefore, they are the most robust, and the effects are consistent across studies. The NOAELs/LOAELs from these studies are, therefore, considered protective of the types of effects seen in other studies.

3.1.1 Dermal Absorption (DA)

There are no acceptable dermal toxicity studies on nanosilver available to EPA. In the absence of any such dermal toxicity studies, the available human *in vivo* study (indicating absorption of nanosilver is 6.7%) (MRID 49052005) and the *in vitro* data (indicating absorption of nanosilver from intact and abraded human skin is substantially below 0.1%) provide scientific support for setting a conservative DA of 6.7% for the nanosilver that might break away from the NSPW Nanosilver. EPA has determined that the dermal toxicity for the nanosilver in NSPW Nanosilver will be evaluated using the oral POD of 30 mg/kg/day and a DA of 6.7%. In 2011, EPA used a

[4] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-considering-and-using-open-literature

DA of 0.1% based on a human clinical study by Moiemen *et al*. (2011) which evaluated the dermal toxicity of the nanosilver in HeiQ AGS-20 (U.S. EPA, 2011a). EPA reviewed this study again as part of this assessment and now concludes that the 0.1% DAF was an underestimate because it was based on the Moiemen *et al*. (2011) study, which only reported silver concentrations in the blood of patients. The 6.7% DA is based on the observational study by Wan *et al*. (1991), which reported silver concentration in serum as well as that eliminated through urine.

The estimated DA of 6.7% is based on application of 500 mg of silver to damaged skin while significantly less silver and nanosilver are used to preserve materials. For example, the maximum dermal exposure to nanosilver from HeiQ AGS-20 treated textiles was 0.17 mg/day (EPA, 2011) as compared to 0.0165 mg/day for NSPW Nanosilver-treated textile (EPA, 2013). Therefore, the concentration of silver in the Wan *et al*. (1991) study was approximately 3,000 to 30,000 times greater than those for materials treated with nanosilver.

Table 1: Toxicity Endpoints and Points of Departure for NPSW Nanosilver

Exposure Scenario	Point of Departure (POD)	Uncertainty Factors	Target MOE for Risk Assessment	Study and Toxicological Effects
Incidental Oral (short-term)	NOAEL = 30 mg/kg/day	$UF_A = 10$ $UF_H = 10$	100	Kim *et al*. (2008) MRID 49052005 LOAEL = 300 mg/kg/day based on increases in alkaline phosphatase and cholesterol, changes in hematology accompanied by histopathological evidence of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein).
Incidental Oral (intermediate-term)	LOAEL = 30 mg/kg/day	$UF_A = 10$ $UF_H = 10$ $UF_L = 3$	300	Kim *et al*. (2010) MRID 49052006 LOAEL = 30 mg/kg/day based on histopathological evidence of liver toxicity (bile-duct hyperplasia with focal, multifocal, or lobular necrosis) in both males and females.
Dermal (short-term)	NOAEL = 30 mg/kg/day DAF = 6.7%	$UF_A = 10$ $UF_H = 10$	100	Kim *et al*. (2008) MRID 49052005 LOAEL = 300 mg/kg/day based on increases in alkaline phosphatase and cholesterol, changes in hematology accompanied by histopathological evidence of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein).
Dermal (intermediate-term)	LOAEL = 30 mg/kg/day DAF = 6.7%	$UF_A = 10$ $UF_H = 10$ $UF_L = 3$	300	Kim *et al*. (2010) MRID 49052005 LOAEL = 30 mg/kg/day based on decreases in reticulocyte count and right kidney weights in females, and minimal bile duct hyperplasia in

Exposure Scenario	Point of Departure (POD)	Uncertainty Factors	Target MOE for Risk Assessment	Study and Toxicological Effects
				male and female rats.
Cancer (oral, dermal, inhalation)	Significant human exposure over a considerable portion of the human lifespan (which is significant in terms of frequency, time, duration, and/or magnitude of exposure) is not expected			

UF = Uncertainty Factor NOAEL = No Observable Adverse Effect Level. LOAEL = Lowest Observable Adverse Effect Level. MOE = Margin of Exposure. LOC = Level of Concern.

3.2 Food Quality Protection Act (FQPA) Safety Factor for Infants and Children

There is no food use for Polyguard at this time, and therefore, no FQPA Safety Factor and no Federal Food, Drug, and Cosmetic Act (FFDCA) aggregate exposure analysis addressing potential NSPW Nanosilver residues is required.

3.2.1 Uncertainty Factors (*i.e.*, Target MOEs) Used for Risk Assessment

The target margin of exposure (MOE) is based on uncertainty factors. There are two standard uncertainty factors that account for potential interspecies extrapolation and intraspecies variation. The first is a 10-fold uncertainty factor (UF_A) assigned to account for extrapolation of laboratory animal data to humans (interspecies). The second is a 10-fold uncertainty factor (UF_H) assigned to account for variations in susceptibility within the human population (intraspecies).

Target MOE: 10 (UF_A) × 10 (UF_H) = 100

The target MOE of 100 is for evaluating the short-term exposure (<30 days) continuous daily oral exposures to NSPW Nanosilver because the oral POD was based on a 28-day oral toxicity study by Kim *et al*., 2008.

The oral POD that was based on the LOAEL from the 90-day oral toxicity study by Kim *et al*. (2010) has an additional 3-fold uncertainty factor (UF_L) to account for the lack of NOAEL. The target MOE for evaluating intermediate-term exposure (1 to 6 months) continuous daily oral exposures to NSPW Nanosilver is:

Target MOE (intermediate-term oral): 10 (UF_A) × 10 (UF_H) × 3(UF_L) = 300

The margin of exposure (MOE) is used to determine if exposure to a chemical can be expected to cause an adverse effect. The MOE is calculated by dividing the POD by the estimated daily dose to which humans will be exposed as expressed by the following:

MOE = POD / Daily Dose

After calculating a MOE from the POD and daily dose, EPA evaluates the risk from exposure to a pesticide by comparing the calculated MOE to a target MOE (U.S. EPA, 2002). If a calculated MOE is **equal to or greater** than a target MOE, EPA may conclude that exposure to the pesticide is unlikely to pose a risk concern and therefore will not cause unreasonable adverse effects for that specific exposure scenario. If a calculated MOE is less than a target MOE, then EPA may have a risk concern. However, the MOE analysis is not the only factor EPA uses when determining if there is a risk concern from exposure to a pesticide. EPA also considers other scientific evidence in a weight of evidence evaluation such as the severity of toxic effects, the controls used to minimize exposures, and the population exposed to the pesticide. A risk concern is not the equivalent of a determination that the potential risk constitutes an unreasonable adverse effect. Rather, where EPA finds a risk concern, EPA will generally: (1) require protective measures or use restrictions to mitigate the risk; (2) further refine its risk assessment analysis, particularly if conservative assumptions were used to produce the initial assessment; or (3) explicitly analyze any potential benefits of the pesticide to determine whether, on balance, those benefits outweigh the identified risk.

There are multiple methods available for determining risk assessment metrics for nanoparticles other than mass (such as particle number or surface area). At this time, EPA's MOE approach for nanosilver uses continues to use the mass-based metrics, both for determining the POD and for calculating exposure.

3.3 Dietary and Drinking Water Assessment

There is no direct or indirect dietary exposure expected from the textile use of Polyguard which contains NSPW Nanosilver.

Although drinking water exposure may occur after down-the-drain release of wash water, the Agency believes overall drinking water exposure to be minimal because the textiles are designed to maintain NSPW Nanosilver embedded within their fibers. Likewise, leached product is not expected to concentrate in any geographic area as use would likely be spread throughout the United States and any NSPW Nanosilver or its nanosilver released to surface water would be removed by gravitational sedimentation and adsorption.

3.4 Residential Exposure from Nanosilver as an Active Ingredient

EPA expects consumer exposures to NSPW Nanosilver, the silver ions released from NSPW Nanosilver, and the nanoparticles that break away from NSPW Nanosilver could potentially occur during incidental oral and dermal exposure to textiles treated with Polyguard.

Polyguard containing NSPW Nanosilver is proposed for use to be mixed into polymer and polymer-based products to suppress the growth of bacterial, algae, fungus, mold, and mildew, which cause odors, discoloration, stains, and deterioration of textiles. Because textiles incorporating NSPW Nanosilver could be subsequently used to manufacture clothing worn by children, it is assumed that children will be exposed to textiles containing NSPW Nanosilver.

In the 2015 Registration Decision for NSPW-L30SS, three age groups of children were assessed. These included a one-year-old toddler with a body weight of 10 kg, a 1- to <2-year-old toddler with a body weight of 11.4 kg and 3-year-old toddler with a body weight of 15 kg. For the purposes of this assessment, however, only the 1- to <2-year old toddler is used, in order to be consistent with the 2012 Standard Operating Procedures (SOPs) for Residential Pesticide Exposure Assessments (U.S. EPA, 2012b). The Impregnated Materials section of these SOPs (Section 9) recommends that the 1- to <2-year-old child, with a body weight of 11.4 kg, be used to represent children exposed to carpets or textiles treated with pesticides. The Indoor Environments section of the SOPs (Section 7) also recommends that the 1- to <2-year-old child be used to represent children exposed to pesticides applied to floors and carpets.

Additional information regarding the relevant lifestages for residential pesticide exposure scenarios is included in Appendix A (Health Effects Division Residential Standard Operating Procedures "Index Lifestage" White Paper) of the 2012 Residential SOPs (U.S. EPA, 2012b). This appendix includes an analysis of the developmental milestones relevant to oral and dermal exposure behaviors that occur during each lifestage ranging from birth to <3 months, 3 to <6 months, 6 to <12 months, 12 to <24 months, 2 to <6 years and 6 to <11 years. Based on this analysis, it was concluded that "the 1- <2-year-old lifestage represents the most appropriate index lifestage for children for most of the exposure scenarios".

3.4.1 Textile Leaching Data Used for Residential Exposure Assessment

Leaching studies are required to determine the amount and form of silver that consumers will be exposed to when in contact with textiles incorporating Polyguard. These studies typically involve immersing products incorporating nanosilver in biological fluids such as simulated saliva solutions for extended periods of time at physiological temperatures (*i.e.*, 98.6° F or 37°C) and measuring the amount and form of silver released to those fluids. A textile leaching study was submitted by the registrant using laundry detergent and simulated saliva for shirts incorporating NSPW Nanosilver. The study was originally submitted as separate reports (MRIDs 49010201 and 49045301) for the laundry detergent and saliva tests. These two reports were revised to correct errors and combined into one report, which was submitted as MRID 49190801. The report was further revised and submitted as MRID 49224901.

The registrant completed and submitted the modified ISO Colour Fastness test (MRIDs 49019201, 49045301, 49190801, and 49224901) using shirts incorporating NSPW Nanosilver.

The washing tests were conducted on shirts composed of polyethylene terephthalate (PET), which incorporated NSPW Nanosilver into the PET yarn for a final nanosilver content of 0.00262% or 26.2 mg/kg per shirt. For the detergent wash test, a section was cut from each of three shirts and washed separately in 150 mL of distilled water with commercial laundry detergent and 10 hard rubber balls with diameter of 10 mm for 30 minutes at 40 degrees Celsius. The detergent wash was followed by two rinse cycles with deionized water. The concentrations of silver in the wash and rinse water were determined after filtering samples through a 0.45 µm pore size filter using inductively coupled plasma mass spectrometry (ICP-MS). The concentration of silver retained on the filter was also determined by ICP-MS. These samples were acid-digested prior to analysis, and thus, the silver concentrations are for total silver content and do not distinguish between silver ions, nanosilver, or NSPW Nanosilver.

Although the NSPW Nanosilver wash test study reported an ICP-MS LOD of 0.0094 µg/L for aqueous samples and 0.94 µg/kg for filter samples, EPA determined that the LOD for aqueous samples was 10 µg/L and 1000 µg/kg for solid samples. EPA determined these LODs based on a statistical analysis of seven ICP-MS calibration curves and the analysis results from 100 µg/L quality control samples. The concentration of silver in the wash and rinse water from the detergent wash test for three shirt sections was below the analytical LOD of 10 µg/L (Table 2). The concentration of silver retained on 0.45 µm pore size filters was also less than the analytical LOD of 1,000 µg/kg.

Table 2: Concentration of Silver Released from Shirts Incorporating NSPW Nanosilver

Number of Shirt Sections	Wash Medium	Concentration of Silver	
		Wash/Rinse Water	0.45 µm Pore Size Filter (Particles with Diameters >0.45 µm)
3	Distilled Water with Detergent	< 10 µg/L	< 1,000 µg/kg
9	Simulated Human Saliva	< 10 µg/L	< 1,000 µg/kg

The simulated saliva tests were conducted using three sections from each of three shirts and washed separately in 150 mL of simulated human saliva with 10 hard rubber balls with diameter of 10 mm for 45 minutes at 40 degrees Celsius. The concentration of silver in the simulated saliva was determined after filtering through a 0.45 µm pore size filter using ICP-MS where the concentration of silver in the saliva for nine shirt sections was below the analytical LOD of 10 µg/L (Table 2).

The concentration of silver retained on filters was less than the analytical LOD of 1,000 µg/kg. Given that none of the silver concentrations in detergent or saliva solutions were above 10 µg/L and none of the filters contained silver at a concentration above 1,000 µg/kg, EPA evaluated the amount of silver released from textiles incorporating NSPW Nanosilver by replacing the non-

detected values with half the LOD, 5 µg/L for liquid samples and 500 µg/kg for solid samples (U.S. EPA, 2000). The amount of silver released from textiles was calculated based on the volume of detergent and rinse water and saliva along with the mass of the 0.45 µm filters used for each test (Table 3).

Table 3: Amount of Silver Released from Shirts Incorporating NSPW Nanosilver

Wash Medium	Volume/Mass	Concentration	Amount of Silver (µg)	Total Silver Potentially Released	
				(µg)	%
Distilled Water with Detergent	190 mL wash/rinse water	5 µg/L[B]	0.95	1.05	1.6
	0.2 g filters[A]	500 µg/kg[B]	0.1		
Simulated Human Saliva	150 mL saliva	5 µg/L	0.75	0.81	0.9
	0.12 g filter	500 µg/kg	0.06		

A. There was a 0.1 g filter used for the detergent wash and another 0.1 g filter for the rinse.
B. Set to half the LOD.

The initial amount of silver in textiles washed with distilled water and detergent was 66.54 µg; therefore, the amount of silver released during the detergent wash was:

Silver Released in Detergent Wash = (1.05 µg/66.54 µg) x 100 = 1.6%

The initial amount of silver in textiles washed with simulated human saliva was 94.9 µg; therefore, the amount of silver released during the saliva wash was:

Silver Released in Saliva Wash = (0.81 µg/94.9 µg) x 100 = 0.9%

The value of 1.6% is used in evaluating releases to the environment from wash water, and the value of 0.9% is used in calculating oral and dermal exposures to textiles incorporating NSPW-Nanosilver. Since these releases were determined using concentrations that were below the ICP-MS LOD, the form of silver (ions released from Polyguard, NSPW Nanosilver in Polyguard, or nanosilver particles that might break away from Polyguard) is unknown. EPA assumes the form of silver is identical to the nanosilver present in NSPW Nanosilver in the absence of further information.

The results of these studies demonstrate that PET shirts which incorporate NSPW Nanosilver at 26.2 µg/kg of nanosilver do not release silver at concentrations above the analytical LOD. The ISO Colour Fastness test is thought to represent aggressive washing conditions with one wash cycle representing up to five domestic or commercial laundering cycles when the multiple test is employed. The amount of silver released during one ISO Colour Fastness test is believed to exceed the daily exposure to nanosilver from a treated textile because the ISO Colour Fastness

test involves immersing the textile in water containing detergents or simulated human saliva and hard rubber balls followed by mechanical agitation for 30 to 45 minutes. Thus, results from studies which are based on the ISO Colour Fastness test are used to determine the daily dose of nanosilver for children who chew and mouth, adults who wear, and workers who manufacture items from nanosilver-treated textiles, even though this likely overestimates the daily dose of nanosilver.

3.4.2 Consumer Margins of Exposure to Textiles Incorporating NSPW Nanosilver

EPA expects that consumers will be exposed to textiles incorporating Polyguard which contains NSPW Nanosilver by the dermal and incidental oral exposure routes.

Consumer Dermal Exposures to Textiles

The dermal exposure to textiles incorporating NSPW Nanosilver was calculated using the following:

Dermal Exposure = Amount of NSPW Nanosilver in Textile × Cloth Density × Surface Area Exposed × Transfer Efficiency

Where:

- The textile incorporating NSPW Nanosilver contains 30 mg/kg nanosilver.
- The cloth density is 10 mg/cm^2 based on the density of mixed cotton and synthetics. This value is a standard assumption used in OPP risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005).
- The total surface area is 5,300 cm^2/day for a 1- to < 2-year-old (U.S. EPA, 2011b).
- The cloth-to-skin transfer efficiency was based on the amount of silver released during the leaching study, which was 0.9% based on one-half the LOD (see Table 3).

The dermal dose was calculated from the dermal exposure using the following:

Dermal Dose = Exposure × Dermal Absorption / Body Weight

Where:

- Exposure is determined in the calculation above.
- The dermal absorption (DA) is 6.7% (see Section 4.2.3).
- The body weight of a child is 11.4 kg between 1 and <2 years, (U.S. EPA, 2012b).

The MOE in Table 4 for dermal exposures was calculated from the dermal dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim *et al*., 2008) and

the LOAEL from a 90-day oral toxicity study (Kim *et al*., 2010). The MOE is 370,000 and is well above the target MOEs of 100 and 300 for short and intermediate term exposures, respectively, which means that the risks are not of concern.

Table 4: Dermal MOEs for Textiles Incorporating NSPW Nanosilver

Age of Child	Application Rate (mg/kg)	Cloth Density (mg/cm^2)	Surface Area Exposed (cm^2/day)	Cloth-to-Skin Transfer Efficiency	Exposure[A] (mg/day)	Dose[B, E] (µg/kg/day)	MOE[C,D]
1 to <2 years	30	10	5,300	0.9%	0.014	0.082	370,000

A. Exposure = Application Rate × Cloth Density × Surface Area Exposed × Cloth-to-Skin Transfer Efficiency
B. Dose = [Exposure (mg/day) × 1,000 µg/mg × DA (6.7%)] / Body Weight (11.4 kg)
C. MOE = [POD (30 mg/kg/day) × 1,000 µg/mg] / Daily Dose (µg/kg/day)
D. Target MOE is 100 for short-term exposures and 300 for intermediate-term exposures.
E. In the 2015 Registration Decision for NSPW Nanosilver, the dose was calculated based on body weight for three separate age groups. The current assessment uses, the body weight of the 1 to <2 year-old child (11.4 kg) to be consistent with the 2012 SOPs for Residential Pesticide Exposure Assessment (US EPA, 2012b).

Consumer Incidental Oral Exposures to Textiles

Incidental oral exposures were calculated using the following:

Incidental Oral Exposure = Amount of NSPW Nanosilver in Textile × Cloth Density × Surface Area Mouthed × Saliva Extraction Efficiency

Where:

- The textile incorporating NSPW Nanosilver contains 30 mg/kg nanosilver.
- The cloth density is 10 mg/cm^2 based on the density of mixed cotton and synthetics. This value is a standard assumption used in OPP risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005)
- The surface area of fabric that is mouthed by a toddler per day is assumed to be 100 cm^2 (~16 in^2), which represents an estimate, for example, of the area of blanket or shirt sleeve.
- The nanosilver saliva extraction efficiencies for mouthing fabric are based on the results of the leaching study, which was 0.9% based on one-half the LOD (see Table 3).

The incidental oral dose was calculated from the incidental oral exposure using the following:

Incidental Oral Dose = Exposure / Body Weight

Where:

- Exposure is determined in the calculation above.
- The body weight of a child is 11.4 kg between 1 and <2 years (U.S. EPA, 2012b).

The MOE in Table 5 for incidental oral exposures was calculated from the incidental oral dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim *et al*., 2008) and the LOAEL from a 90-day oral toxicity study (Kim *et al*., 2010). The MOE is 1,200,000 and is well above the target MOEs of 100 and 300 for short- and intermediate-term exposures, respectively, which means that the risk for short- and intermediate-term exposure to children who mouth textiles incorporating NSPW Nanosilver from Polyguard is not of concern.

Table 5: Incidental Oral MOES for Textiles Incorporating NSPW Nanosilver

Application Rate (mg/kg)	Cloth Density (mg/cm^2)	Surface Area Mouthed (cm^2/day)	Saliva Extraction Efficiency	Exposure[A] (mg/day)	Dose[B] (µg/kg/day)	MOE[C, D]
30	10	100	0.9%	0.00027	0.024	1,200,000

A. Exposure = Application Rate × Cloth Density × Surface Area Mouthed × Saliva Extraction Efficiency
B. Dose = [Exposure (mg/day) × 1,000 µg/mg] / Body Weight (11.4 kg)
C. MOE = [POD (30 mg/kg/day) × 1,000 µg/mg] / Daily Dose (µg/kg/day)
D. Target MOE is 100 for short-term exposures and 300 for intermediate-term exposures.

Consumer Combined Dermal and Incidental Oral Exposure

The following analysis is for children who are simultaneously exposed to nanosilver via the incidental oral and dermal routes of exposure while wearing and mouthing textiles incorporating NSPW Nanosilver. The combined daily dose was calculated by adding the daily oral and dermal doses using the following:

Combined Dose = Dermal Dose to Textiles Incorporating NSPW Nanosilver + Incidental Oral Dose to Textiles Incorporating NSPW Nanosilver

Where:

- Dermal Dose to Textiles Incorporating NSPW Nanosilver is from Table 4; and
- Incidental Oral Dose to Textiles Incorporating NSPW Nanosilver is from Table 5.

The oral and dermal daily doses can be combined because they are evaluated using the same POD of 30 mg/kg/day. The combined MOE in Table 6 is 270,000 and is well above the target MOEs of 100 and 300 for short and intermediate terms exposures, respectively, which means that the risk for short- and intermediate-term combined exposure to children is not of concern.

Table 6: Combined MOEs for Textiles Incorporating NSPW Nanosilver

Age of Child	Dermal Dose (µg/kg/day)	Incidental Oral Dose (µg/kg/day)	Combined Dose[A] (µg/kg/day)	Combined MOE[B,C]
1 to <2 years	0.082	0.024	0.11	270,000

A. Combined Dose = Sum of the Incidental Oral and Dermal Doses (µg/kg/day) for Textiles
B. Combined MOE = [POD (30 mg/kg/day) × 1,000 µg/mg] / Dose (µg/kg/day)
C. The target MOE is 100 for short-term exposure and 300for intermediate-term exposures.

3.5 Aggregate Exposure/Risk Characterization

In the Federal Food, Drug, and Cosmetic Act (FFDCA), Congress specified that, to establish an acceptable level of a given pesticide's chemical residue that could be found in or on food products, EPA must determine that "there is a reasonable certainty that no harm will result from aggregate exposures to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." Furthermore, when enacting this provision of the FFDCA, Congress also amended FIFRA's definition of unreasonable adverse effects. Specifically, Congress redefined unreasonable adverse effects to include "a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under" the FFDCA. In other words, Congress explicitly required the consideration of aggregate exposures for registration decisions under FIFRA for food-use pesticides but chose not to similarly alter the statutory requirements for non-food-use pesticides.

In addition to the consumer dermal, inhalation and incidental oral exposures discussed above in Section 3.9.2, aggregate assessments can also include other sources of exposure such as to NSPW Nanosilver in food and drinking water and to other nanosilvers in the market place that are identical to the nanosilver in NSPW Nanosilver. There are no anticipated food exposures to NSPW Nanosilver, since the pesticide label for NSPW Nanosilver states that it is only for non-food contact use. Neither the nanosilver that might break away from NSPW Nanosilver nor NSPW Nanosilver is anticipated to enter drinking water because any particulates released to surface water would be removed by gravitational sedimentation and adsorption (see Section 3.8).

The only other pesticide product registered as containing nanosilver is HeiQ AGS-20 (U.S. EPA, 2011a). The nanosilver in HeiQ AGS-20 has different size ranges and surface coatings than the nanosilver in Polyguard, and EPA has not determined that AGS-20 and NSPW Nanosilver (Polyguard) contain the same active ingredient or present exposures that should necessarily be aggregated.

3.6 Cumulative Exposure/Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as

to NSPW Nanosilver and any other substances and NSPW Nanosilver does not appear to produce a toxic metabolite produced by other substances. For the purposes of this action, therefore, EPA has not assumed that NSPW Nanosilver has a common mechanism of toxicity with other substances. In 2016, EPA's Office of Pesticide Programs released a guidance document entitled, *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis* (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework). This document provides guidance on how to screen groups of pesticides for cumulative evaluation using a two-step approach beginning with the evaluation of available toxicological information and if necessary, followed by a risk-based screening approach. This framework supplements the existing guidance documents for establishing common mechanism groups (CMGs)[5] and conducting cumulative risk assessments (CRA)[6]. During registration review, the Agency will utilize this framework to determine if the available toxicological data for NSPW Nanosilver suggests a candidate CMG may be established with other pesticides. If a CMG is established, a screening-level toxicology and exposure analysis may be conducted to provide an initial screen for multiple pesticide exposure.

3.7 Occupational Exposure/Risk Characterization

Occupational handler exposures are not expected during the handling of master batches containing NSPW Nanosilver because they are in the form of plastic beads or pellets.

4 ENVIRONMENTAL RISK ASSESSMENT

EPA anticipates the following substances could enter the environment through leaching of textiles incorporating Polyguard:

1) Silver ions released from Polyguard
2) NSPW Nanosilver in Polyguard; and/or
3) Nanosilver particles that might break away from Polyguard.

There are no studies available to characterize the environmental fate of NSPW Nanosilver, but there are studies available in the scientific literature for nanosilver. Since nanosilver may be released from NSPW Nanosilver, EPA has considered the scientific literature studies on nanosilver fate and ecotoxicity relevant to NSPW Nanosilver. The following sections cover the environmental fate of nanosilver, the environmental hazards posed by silver and nanosilver, and the potential risk to aquatic species from nanosilver. Because differences in formulations from one nanosilver to another are likely to affect biological activity and fate, EPA has attempted to

[5] *Guidance For Identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity* (USEPA, 1999)
[6] *Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (USEPA, 2002)

be cautious in how it has used information about other nanosilvers from the literature in assessing risks from NSPW Nanosilver.

4.1 Environmental Fate

The registrant has not conducted any studies to characterize the environmental fate of NSPW Nanosilver or other particles that could be released during leaching or disposal of textiles incorporating NSPW Nanosilver. However, studies have been submitted on the physical-chemical properties of NSPW Nanosilver, including particle size, zeta potential, and dissolution kinetics, and they are discussed below. These studies provide some information on the behavior of NSPW Nanosilver in water, including colloidal stability (or aggregation) over time, surface charge, and zeta potential-pH dependence. EPA is relying on this submitted information as well as studies available in the scientific literature, discussed in the following section, as the basis for determining the fate of nanosilver in the environment. The environmental fate data requirements and the rationales for waiving them are listed in the 158w data requirements table (Appendix E).

NSPW Nanosilver particles consist of silica core particles dotted on the surface with silver particles (which are bound by a thiolate bond). A product chemistry study (MRID 50649402), which shows samples at 9 days old, indicated the size of the silica particles to be between 20 nm and 40 nm (most as ~20 nm), while the silver particles were 2-3 nm wide. This is consistent with the registrant's statements claiming that the overall diameter of the NSPW Nanosilver particles is 30-50 nm, with silver particles in the 2-3 nm range (Appendix B). The effects of temperature and duration on morphology of the particles (*i.e.*, artificial aging) were also investigated, and no significant (distinguishable) change in morphology was found (MRID 50649402).

When placed in solution, NSPW Nanosilver is not stable over time, and because of this instability, it is converted to (solid) master batch as soon as possible (Appendix C). MRID 50699402 (supersedes MRID 49941901) shows the particles at 20 days old, in which the silver particles had an average diameter of 12.9 nm (range of 4-50 nm) and the silica particles had an average diameter of 19.2 nm (range of 5-39 nm). The size of the silica particles was consistent with that of the 9-day-old samples in MRID 50649402, but the silver particles grew over time and were roughly 5 times larger (on average) at 20 days than at 9 days. These silver particles might have grown over time (due to Ostwald ripening) and/or aggregated with other particles to form larger particles. Because the NSPW Nanosilver solution is quickly converted to master batch and MRID 50649402 shows samples with minimal aggregation compared to MRID 50699402, the 9-day-old samples in MRID 50649402 are considered representative of the morphology of NSPW Nanosilver both at manufacture and for any potential exposure to the environment and humans.

Clusters of particles in the TEM images, large hydrodynamic sizes relative to physical sizes, high degree of polydispersity (0.4-0.8, >0.3), and low zeta potential (magnitude <30 mV) (MRIDs

50649402, 50699402, and 50617302) all indicate a strong tendency for NSPW Nanosilver to aggregate/agglomerate. Precipitation/sedimentation was also reported by the testing laboratories. Hydrodynamic sizes measured by DLS vary widely, ranging from approximately 28-190 nm (average of 44 nm) after 1 hour of sonication (Appendix C) to peak 1 averages of 961-1,816 nm after 0-48 hours of orbital shaking (MRID 50617302). When pH was increased from 4.3 to 10, the zeta potential became more negative from -5 mV to -17 mV, indicating a weak negative surface charge on NSPW Nanosilver as well as a weak dependence of zeta potential on pH (MRID 50617302). The negative surface charge on the NSPW Nanosilver particles is due to the high oxygen content from silica. NSPW Nanosilver is of acidic nature with pH values ranging from 1.81 ± 0.02 (MRID 47828913) to 2.5-3.5[7]. The isoelectric point (IEP) is expected to be in the acidic range, as the zeta potential was at minimum (or close to zero) at pH ~5. Despite the very small particle size, the specific surface area is small at 18.2688 m^2/g (MRID 50617302) and is most likely due to the blocking of silica pores by silver particles (Zienkiewicz-Strzałka *et al*., 2017; Zienkiewicz-Strzałka *et al*., 2018). The high degree of aggregation/agglomeration (including sedimentation) and/or possibly low porosity of the nanoparticles may contribute to the small surface area as well.

The elemental composition of NSPW Nanosilver active ingredient is 60.7% oxygen, 37.0% silicon, 1.8% silver, and 0.5% sulfur (MRID 50699402). The oxygen/silicon ratio (1.6:1) is generally consistent with the stoichiometric silica (2:1). However, the 1.8% silver composition is higher than the 1% stated on the label. The registrant explained that the 1% concentration on the label reflects the amount of silver (from the silver nitrate reagent) *prior* to the synthesis of NSPW Nanosilver and is not the amount of silver in the final product (Appendix C). It is likely that because of sampling variation (likely high due to significant aggregation/agglomeration) as well as drying preparation, there was more silver and/or larger silver particles in some samples and less silver in others and that the sample used in elemental composition analysis contained a higher amount.

NSPW Nanosilver is essentially insoluble (MRID 47828917). However, the silver particles were shown in the dissolution study (MRID 50617302) to dissolve into ions to a certain extent, after which the suspension entered a kinetic equilibrium and the released ions subsequently re-formed new (secondary) nanoparticles. Similar dissolution and re-formation behavior of silver nanoparticles has been observed in published literature (Lee *et al*., 2011; Wildt *et al*., 2015; Azodi *et al*., 2016; Zhang *et al*., 2017). Of the total silver in the NSPW suspension, approximately 25% is ionic, and the remaining 75% is particulate (MRID 50617302).

[7] Nanosilva, LLC. Material Safety Data Sheet for NSPW-L30SS.

4.1.1 Nanosilver

The fate of silver nanoparticles (AgNPs) in the environment remains poorly understood, as there are many factors influencing the behavior and toxicity of AgNPs. Factors include properties of AgNPs (*e.g*., size, shape, coating, and charge) as well as environmental conditions (*e.g*., organic matter content, soil texture, ionic composition, and pH) (Grün *et al*., 2019). AgNPs released into soil/sediment and water may remain as nanoparticles, dissolve into silver ions, adsorb to molecules or cells, and/or aggregate/agglomerate. The resulting form in turn affects the silver's mobility as well as its toxicity and bioavailability.

Both protons and dissolved oxygen are required for silver dissolution (Liu & Hurt; 2010). Presence of organic matter in soils and aquatic environments inhibits dissolution, due to the adsorption of organic matter to the surface of nanosilver (Klitzk *et al*., 2014). The rate at which nanosilver transforms into ionic silver determines the length of time that these particles will reside in the environment. Although there are studies reporting that nanosilver will completely transform into ionic silver within six days after being dispersed into air-saturated deionized water (Liu & Hurt, 2010), these results are only for one form of nanosilver (2-8-nm citrate-stabilized AgNPs) and are under conditions which are not representative of the environment. In the environment, nanosilver is likely to complex with naturally occurring anions such as chloride and sulfide or natural organic matter such as humic acids, which will significantly delay the rate at which nanosilver transforms into ionic silver. For example, Choi *et al.* (2009) provided spectroscopic evidence showing that nanosilver (average size of 15 ± 9 nm, in 0.06% polyvinyl alcohol solution) reacts with a stoichiometrically equivalent amount of sulfide to produce stable silver-sulfide complexes, which were shown by Levard *et al.* (2011) to dramatically reduce the dissolution rate of AgNPs. These stabilized nanosilver complexes are likely to partition to sediments, rather than remain suspended in water, due to gravitational settling and coagulation processes (FIFRA SAP, 2009, p. 19). Likewise, nanosilver is anticipated to partition to biosolids during wastewater treatment but may also be released in the effluent. Thus, there is potential for nanosilver to reside or persist in the environment where these particles are most likely to be associated with sediments. Grün *et al*. (2019), Ramskov *et al*. (2015), and Rajala *et al*. (2017) demonstrate that sediments act as a major sink for AgNPs, which eventually release silver ions into soils and aquatic environments, resulting in potential exposure to soil microorganisms and sediment-dwelling organisms.

4.1.2 Silver Ions

Ionic silver(I), Ag^+ ions, typically has low concentrations in natural waters, in the nanogram per liter range, due to its reactivity with chloride, sulfides, and natural organic matter (Andren & Armstrong, 1999). Silver(I) can readily react with sulfide ions and organic materials bearing thiol groups. Silver sulfides are insoluble, and in sulfide-rich natural waters, the formation of insoluble sulfides serves to immobilize silver (Morel, 1983). Thiol groups in aquatic sediments

also contribute to the removal of silver(I) from the aqueous phase (Morel, 1983). As with nanosilver, ionic silver is found in sediments and associated with biosolids in wastewater treatment plants (WWTPs).

4.1.3 Impacts to Wastewater Treatment/Septic Systems

There is the potential for nanosilver that might be released from textiles incorporating Polyguard to reach publicly owned wastewater treatment and privately-owned septic systems where they will most likely complex with sulfide and partition to biosolids (Kaegi *et al.*, 2011). Once entrained in the biosolids, the nanosilver could serve as a "sink" or long-term source of ionic silver and could potentially adversely affect microorganisms that are vital to wastewater treatment processes. Reports in the scientific literature regarding the impact of nanosilver on wastewater treatment systems have been contradictory. For example, nanosilver was reported to inhibit nitrification in the range of 50% (Choi & Hu, 2009a) to 84% (Choi & Hu, 2009b), based on a reduction in oxygen uptake rate in simulated wastewater sludge. However, Burkhardt *et al.* (2010) found no impact to nitrification at nanosilver dosages of 1 mg/L, the same dosage that Choi and Hu (2009a & 2009b) reported as inhibitory in municipal wastewater sludge. A third group independently determined that nanosilver at concentrations from 0.5 to 1.5 mg/L had no detectable effect on the ability of the wastewater bacteria to biodegrade organic material, as measured by chemical oxygen demand (COD) (Wang *et al*., 2012). More recent work by the Hu group reported that nanosilver at concentrations of up to 40 mg/L had negligible impact on anaerobic digestion and methanogenic organisms (Yang *et al*., 2012).

While there are reports suggesting the potential for nanosilver to impact wastewater treatment operations, EPA does not anticipate that registering Polyguard will lead to negative impacts to wastewater treatment systems. This conclusion is based on the limited amount of silver released from textiles incorporating NSPW Nanosilver (see Section 3.4.1) and the small volume of nanosilver (*i.e.*, <1,123 kg/yr as estimated in Section 4.3.2) expected to be introduced into commerce from textiles incorporating NSPW Nanosilver.

4.2 Ecological Effects

The registrant conducted a daphnid study (MRID 50617301, updated MRID 50699401) to evaluate the risk to aquatic species from nanosilver particles released during potential leaching or disposal of textiles embedded with NSPW Nanosilver. In order to characterize the environmental risk to NSPW Nanosilver, the Agency is relying on this study as well as silver ion endpoints from within the Agency's database that have been used to support products containing non-nano-sized silver.

4.2.1 NSPW Ecotoxicity Study

The registrant has submitted one acute daphnid study with the test substance NSPW Nanosilver (MRID 50617301, updated MRID 50699401). The laboratory conducting the daphnid study did not have the capacity to perform analytical measurements at the nano scale; therefore, information from the NSPW Nanosilver dispersion and dissolution study (MRID 50617302) was utilized to find a calculated nanosilver concentration. Within the daphnid study, a renewal and a non-renewal study were performed and an EC_{50} for each was determined. The results of this study are found in Table 7.

Table 7: Daphnid Toxicity Data on NSPW Nanosilver

Type of Definitive Test	NSPW Nanosilver EC_{50} (Nominal, product)[1]	Nanosilver EC_{50} (Calculated)[2]
Renewal (at 24 and 48 hrs)	187.5 µg/L NSPW Nanosilver	0.150 µg/L Nano Ag
Non-renewal (at 48 hrs)	180.6 µg/L NSPW Nanosilver	0.144 µg/L Nano Ag

[1] EC_{50}s in study reported as mg/L. 1 mg/L = 1,000 µg/L

[2] Based on the dissolution study (MRID 50617302) and communication with the Registrant, the stock NSPW Nanosilver solution (1 g NSPW / 1,000 mL) contained 26.62% solids of which 0.30% is nano Ag. Therefore, the EC_{50} of Nano Ag (calculated) = Nominal EC_{50} * 0.2662 * 0.0030

4.2.2 Open Literature Nanosilver Ecotoxicity Studies

A literature search was conducted to determine if open literature studies were available for nanosilver ecotoxicity studies. On April 8, 2019, EPA's ECOTOX database was searched with the following keywords: "nanosilver," "nano silver," "nanoparticles," "nano particles," "NSPW," and "silver nanoparticles." No open literature studies were identified within the database.

Additionally, Google Scholar was searched using various terms. The terms "NSPW," "NSPW Nanosilver," and "Nanosilva" came up with no relevant results to ecotoxicity. The terms "nanosilver AND EC50 AND *Daphnia*" and "nanosilver AND EC50 AND *Daphnia* AND OECD" from years 2014 to 2019 produced around 350 citations. The majority of the citations did not include information relevant to the scope of the current risk assessment. The most useful articles included review articles and studies conducted under OECD 202 guidelines.

The review papers comparing multiple taxa of aquatic organisms found that invertebrates were the most sensitive taxa to nanosilver. Additionally, the various review articles and studies conducted under OECD guidelines presented EC_{50} values that were no more acutely toxic than the EC_{50} of NSPW-derived nanosilver calculated in MRID 50617301/50699401 (0.144-0.150

µg/L nanosilver from NSPW). A list of the most relevant literature studies screened are provided in Appendix F.

These conclusions align with the studies presented in the 2015 literature search conducted in support of NSPW (U.S. EPA, 2015) and supports the use of the nanosilver EC_{50} from the NSPW Nanosilver *Daphnia* study within this risk assessment. It should be noted that the endpoints available within the open literature will not be utilized within this risk assessment except as a weight of evidence to confirm *Daphnia* are the most sensitive species of those tested. It is unknown whether the nanoparticles tested were substantially similar in size or structure to NSPW Nanosilver.

4.2.3 Silver Ion Endpoints

EPA has considerable data on the environmental hazards posed by the release of silver ions from silver-based pesticide products that are not nanosized (*i.e.*, silver chloride, silver nitrate, silver sulfide) (Appendix D). The precious metal silver is a trace element found in the Earth's crust and is generally naturally present in surface waters in relatively low concentrations as compared to metals such as copper and zinc. However, it may become toxic to aquatic life at elevated concentrations. Thus, silver concentrations in natural environments, and its biological availability, are important. Naturally occurring concentrations of silver have been reported from about 0.0002 to just over 1 µg/L in freshwater systems (Campbell *et al*., 2002). Elevated concentrations of silver in surface waters have generally been associated with wastewater treatment plant effluent discharges (Bell and Kramer, 1999).

Consistent with previous silver assessments, the silver ion ecotoxicity endpoint used in this assessment is based on the US EPA Ambient Water Quality Criteria for silver (1980; 1987). Water hardness or associated factors are known to influence silver ion toxicity. Therefore, the acute toxicity values for various species were normalized to a water hardness of 50 mg/L (Appendix C). Using this approach, *Daphnia magna* was found to be the most sensitive species with a normalized endpoint of 0.4 µg/L (ppb). Although there are chronic (NOEC) data for *Daphnia*, the chronic endpoints are higher than the acute endpoints. This is attributed to the presence of food in the long-term exposure studies as compared to the acute studies (*i.e.*, food binds to silver rendering it less bioavailable). Likewise, although an acute to chronic ratio (ACR) could be derived from other freshwater invertebrate species (such as the *Hyalella azteca*), the uncertainties surrounding the ratio would be high. Although, a chronic endpoint is, currently, not available, available leaching data show that acute and chronic exposures are expected to be low. Therefore, no chronic data are needed since no chronic risk assessment is planned for this textile use

4.3 Aquatic Exposure Assessment

EPA expects that NSPW Nanosilver in Polyguard, nanosilver particles that might break away from Polyguard, and silver ions released from Polyguard could enter the environment primarily through the leaching of textiles incorporating NSPW Nanosilver. While several textile uses including interior use items such as draperies and upholstery and exterior use items such as tents and awnings could contribute to environmental exposures, items such as T-shirts, which are regularly laundered are considered to have the highest leaching potential. The silver released via leaching during the laundering process could be discharged to the sanitary sewer system leading to publicly owned wastewater treatment and privately-owned septic systems, also known as the down-the-drain discharge scenario. Once NSPW Nanosilver, silver ions, or nanosilver reach wastewater treatment and septic systems, they will most likely complex with sulfide and partition to biosolids. However, some fraction of the silver compounds will reach surface water and may potentially impact aquatic organisms.

As stated in Section 3.4.1, silver (in all forms) was not found above the analytical LOD leaching from shirts incorporating NSPW Nanosilver using the ISO Colour Fastness test (MRIDs 49019201, 49045301, 49190801, 49224901). EPA does not expect any silver ions released to cause unreasonable adverse effects to the environment based on risks estimated for other registered products that release silver ion (U.S. EPA, 1993). Therefore, to evaluate the impacts on surface water from the leaching of NSPW Nanosilver from textiles, within the following assessment EPA assumes that nanosilver is the only silver compound released from NSPW Nanosilver.

4.3.1 Aquatic Risk Quotient Methodology

EPA uses a Risk Quotient (RQ) approach to assess impacts to surface water, which is similar to the MOE used for the human health risk assessment. The RQ is used to compare toxicity from potential environmental exposure by dividing a point estimate of exposure by a point estimate of effects. This ratio is a simple, screening-level estimate that identifies high- or low-risk situations. In this method, the estimated environmental concentration (EEC) is compared to an effect level, such as an EC_{50}. After the RQ is calculated, it is compared to the Agency's Level of Concern (LOC). A LOC is a policy tool that the Agency uses to interpret the RQ and to analyze potential risk to non-target organisms and the need to consider regulatory action (U.S. EPA, 2011c).

Risks to aquatic organisms associated with the in-service use of preserved textiles was used to screen for aquatic environmental loading of nanosilver from Polyguard and risks from all uses of these materials listed on the label. Based on the results of the screening risk assessment, no further refinement of the environmental loading and risks by in-service use were conducted.

4.3.2 Exposure from Textile Leaching

The concentration of nanosilver in surface water resulting from the use of Polyguard containing NSPW Nanosilver in textiles was calculated using the Down the Drain (DtD) Module of the Exposure and Fate Assessment Screening Tool (E-FAST model, version 2). The following input values were used:

- Mass of silver release per year: 1,123 kg/year.
  - The amount of silver released from textiles incorporating NSPW Nanosilver was derived assuming: 300 million people (approximate U.S. population) purchase one t-shirt incorporating NSPW Nanosilver each year. Each t-shirt weighs 150 grams and contains 0.003% nanosilver by weight silver. Each t-shirt is washed once per week for 52 weeks/yr and releases 1.6% of its silver per wash (based on ½ LOD from textile leaching study. Percent is for all forms of silver).
- Release Rate: Each t-shirt is washed once per week for 52 weeks/yr
- Wastewater Removal Efficiency was set at 85% based on: Blaser *et al*. (2008) found a removal rate ranging from 85 to 99% (silver). Wang *et al*. (2012) reported nanosilver removal of 88% with biomass present.
- Speciation, Agglomeration and Sedimentation in the Surface Water Column: All of the silver released to surface water from a wastewater facility was assumed to be in the nanosilver form and it was assumed that all of the nanosilver was retained in the water column (*i.e*., no removal of released silver from the water column). The model used does not include these fate mechanisms and therefore surface water concentrations are expected be overestimated.
- E-FAST Stream Dilution Factor: 1.0 or 20.1. These values are the 10th and 50th percentile values for the dilution that occurs during one day of lowest stream flow over a ten-year period (1Q10) (U.S. EPA, 2007b).

Table 8: Estimated Surface Concentrations of Nanosilver from Textiles Using E-FAST

WWTP Removal [A]	Stream Dilution	Estimated Surface Water Nanosilver Concentration (µg/L) from DtD Sources
85%	Low Dilution [B]	0.004
	Average Dilution [C]	0.0002

A- Silver removed from wastewater during treatment before discharge to a water body (e.g., lake, river, *etc*.) based on studies by Blaser *et al*. (2008) and Wang *et al*. (2012).
B- 10th Percentile dilution factor for 1Q10 stream flow.
C- 50th Percentile dilution factor for 1Q10 stream flow.

4.4 Ecological Risk Assessment

The down-the-drain modeling results in Table 8 were then divided by the EC_{50}s for nanosilver and silver ions for *D. magna* to obtain acute risk quotients (RQs) presented in Table 9. The effect

level used to calculate the acute RQs was chosen to represent the most sensitive aquatic organism, *D. magna,* and to represent conditions that are representative of surface water in the United States. It should be noted the estimated concentrations calculated within the DtD model did not differentiate between forms of silver leaching from textiles. Therefore, the nanosilver RQ contains the conservative assumption that all the silver released from NSPW Nanosilver was nanosilver. Silver ion RQs were calculated for characterization in order to demonstrate the risk to *D. magna* if an equivalent amount of silver ion from non-nanoparticle sources were released into the environment.

$$RQ = \frac{Estimated\ Exposure}{Endpoint}$$

- The toxicity value for nanosilver: 0.144 µg/L nanosilver based on the 48-hr EC_{50} value for *Daphnia magna* from MRID 50617301 (updated MRID 50699401)
- The toxicity value for silver ion: 0.4 µg/L silver ions based on the normalized LC_{50} value for *Daphnia magna* the Ambient Water Quality Criteria (AWQC) for silver.
- Level of Concern for the RQ: The presumptive acute level of concern (LOC) is 0.05 for listed (*i.e*., endangered or threatened) aquatic animals and 0.5 for non-listed animals.

Table 9: Acute Risk Quotients (RQs) for Nanosilver in Surface Water

WWTP Removal [A]	Stream Dilution	Estimated Concentration (µg/L) from E-FAST	Nanosilver RQ [B]	Silver Ion RQ [B,C]
85%	Low Dilution [D]	0.004	0.028	0.01
	Average Dilution [E]	0.0002	0.0014	0.0005

*The presumptive acute LOC is 0.05 for listed animal species and 0.5 for non-listed animal species.
A- Silver removed from wastewater during treatment before discharge to a water body (e.g., lake, river, *etc*.) based on studies by Blaser *et al*. (2008) and Wang *et al*. (2012).
B- Acute RQ = Surface Water Concentration / EC_{50} for *D. magna*
C- Acute RQ if assuming an equivalent quantity of silver ions were released from textiles
D- 10th Percentile dilution factor for 1Q10 stream flow.
E- 50th Percentile dilution factor for 1Q10 stream flow.

4.4.1 Ecological Risk Characterization

4.4.1.1 Freshwater Organisms

The acute RQ for nanosilver from NSPW Nanosilver for the worst-case, 1Q10 stream flow with low stream dilution, was 0.028 and is below the level of concern for listed and non-listed aquatic invertebrates. This indicates that it is unlikely that the registration of NSPW Nanosilver as a preservative in textiles will lead to adverse effects for listed or non-listed aquatic organisms.

Nanosilver derived from NSPW Nanosilver as well as silver ions are categorized as very highly toxic to freshwater invertebrates. Although the acute EC_{50} for nanosilver derived from NSPW Nanosilver is below that of silver ions, indicating that it may be more toxic to aquatic species than silver ions from non-nano sources, the endpoints are within the same order of magnitude of each other.

It should be noted that the current assessment does not take into consideration long-term (chronic) risks to aquatic organisms because no chronic endpoints are available for NSPW Nanosilver, nanosilver derived from NSPW Nanosilver, nor silver ions derived from NSPW Nanosilver. Although there are chronic silver ion data for *Daphnia*, the chronic endpoints are higher than the acute endpoints potentially due to the presence of food in the long-term exposure studies as compared to the acute studies. While there are no chronic toxicity data for NSPW Nanosilver available, chronic exposure is likely to be negligible for NSPW Nanosilver because the silver-silica-sulfur complex nanosilver is expected to be unstable in water and any chronic exposure would be to silver ions. Further, as stated earlier, based on textile leaching data, exposure to silver ions would be expected to be minimal. Therefore, a chronic assessment is not needed.

4.4.1.2 Estuarine/Marine Organisms

This assessment does not quantitatively assess exposure to estuarine and marine species because the DtD model is appropriate only for use for flowing water (*i.e.*, streams and rivers). Due to dilution within freshwater waterways after release from a WWTP but before release into estuarine/marine ecosystems, exposure of NSPW-derived nanosilver to estuarine/marine organisms are expected to be negligible.

4.4.1.3 Terrestrial Organisms

This assessment does not consider exposure to terrestrial organisms. The use of Polyguard as a material preservative within textiles is expected to result in negligible exposure to terrestrial organisms.

4.5 Summary of Major Risk Presumptions

It is unknown how much of the US textile market will eventually contain Polyguard as a material preservative. Therefore, the current assessment assumes that every person in the US will own one shirt containing 0.003% NSPW Nanosilver as a material preservative, that they will wash it once a week, and 1.6% of the silver it contains (nanosilver, silver, and NSPW nanoparticles combined) will be released with every wash. Likewise, this approach assumes that

the release of NSPW Nanosilver will be equally distributed across wastewater treatment plants (WWTPs) in the US.

The Agency believes that it is taking a conservative approach in its screening-level exposure estimates since (1) the submitted textile leaching study showed no leaching above the level of detection (LOD) and the 1.6% leaching represents exposure to ½ LOD, (2) within E-FAST, the lowest stream flow within a 10-year period (1Q10) was modeled for average and low stream dilution, and (3) the E-FAST model did not account for removal and sorption of nanosilver particles to organic matter within the water column. Even with these conservations, no risks of concern to aquatic species were identified.

4.6 Major Uncertainties and Data Gaps

No studies have been submitted to characterize the environmental fate of NSPW Nanosilver or the other particles that could be released during leaching or disposal of textiles incorporating NSPW Nanosilver. In lieu of this information, EPA has relied on studies available in the scientific literature and studies submitted by the registrant as the basis for determining the fate of nanosilver in the environment.

No sediment ecotoxicity or exposure data are available for NSPW Nanosilver. However, based on the low application rate, the low leaching rate, and the instability of the NSPW Nanosilver particle discussed in Section 4.1, sediment organism exposure to nanosilver and silver ions is expected to be low. No additional sediment data are needed for this textile use.

There is some uncertainty within the endpoint derived from the acute *Daphnia* study because the nominal concentrations were not analytically tested within the study; rather, endpoints were calculated based on information from the NSPW Nanosilver dispersion and dissolution study (MRID 50617302). Both labs experienced precipitation of NSPW Nanosilver from the solution, and it cannot be verified if the concentration of the precipitate was consistent between the labs. That being said, the Agency is confident in the use of the calculated endpoint because (1) the dispersion and dissolution study found the concentration of total silver, silver ion, and nanosilver in solution remained constant over the 48 hours tested, indicating that once in solution, the concentration remains in equilibrium and (2) the endpoints found within the open literature for other nanosilver particles indicate endpoints that are higher than the NSPW Nanosilver study. There is no scientific explanation for why the NSPW Nanosilver nanoparticle structure or product chemistry would produce a more toxic form of silver, which provides confidence that the Agency is taking a conservative approach by relying on the calculated concentration.

No NSPW Nanosilver specific ecotoxicity data beyond the *Daphnia* study have been submitted to the Agency. This brings some uncertainty as to the acute and chronic effects of nanosilver from this product to other organisms. Nonetheless, the current assessment evaluated the acute

risk to the species most sensitive to other types of silver (silver ions, nanosilver from other sources, *etc*.) and found no risks. Therefore, the risk conclusions based on the *Daphnia* data are assumed to be protective of other aquatic organisms and no additional ecotoxicity is required at this time to support the NSPW Nanosilver registration for use as a material preservative in textiles. Likewise, no chronic exposure to NSPW Nanosilver is expected, and therefore, no chronic data or chronic assessment are needed.

5 LISTED SPECIES OF CONCERN

Although NSPW Nanosilver is acutely toxic to aquatic invertebrates, the potential exposures to aquatic organisms (including listed species) are expected to be negligible and risks were determined to not be of concern based on the aquatic analysis. Further, no terrestrial exposure is expected from the use of NSPW Nanosilver in textiles. Therefore, there is no reasonable expectation of risks (*i.e*., direct adverse effects) to non-target organisms. Because direct adverse effects are not expected for any non-target organism (including birds, reptiles, amphibians, mammals, fish, aquatic invertebrates, aquatic plants, terrestrial plants, and terrestrial invertebrates), EPA makes a No Effects (NE) determination for all Federally-listed threatened/endangered species from the proposed textile use of NSPW Nanosilver as described on the Polyguard label. EPA also makes a NE determination for designated critical habitats.

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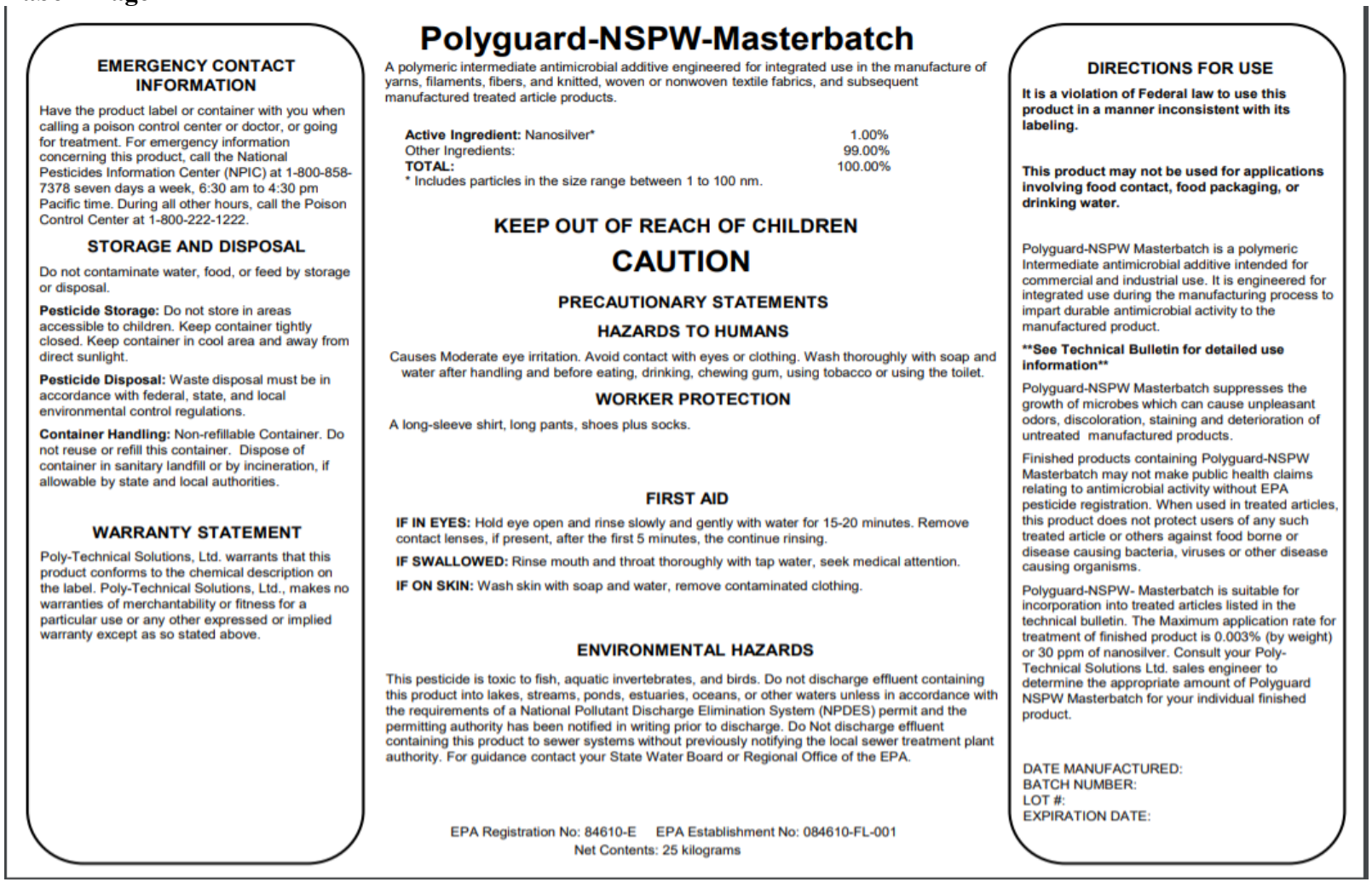
APPENDIX A: Master Label for EPA Reg. No. 84610-E as of 7/12/2018

Label Image



EMERGENCY CONTACT INFORMATION

Have the product label or container with you when calling a poison control center or doctor, or going for treatment. For emergency information concerning this product, call the National Pesticides Information Center (NPIC) at 1-800-858-7378 seven days a week, 6:30 am to 4:30 pm Pacific time. During all other hours, call the Poison Control Center at 1-800-222-1222.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

Pesticide Storage: Do not store in areas accessible to children. Keep container tightly closed. Keep container in cool area and away from direct sunlight.

Pesticide Disposal: Waste disposal must be in accordance with federal, state, and local environmental control regulations.

Container Handling: Non-refillable Container. Do not reuse or refill this container. Dispose of container in sanitary landfill or by incineration, if allowable by state and local authorities.

WARRANTY STATEMENT

Poly-Technical Solutions, Ltd. warrants that this product conforms to the chemical description on the label. Poly-Technical Solutions, Ltd., makes no warranties of merchantability or fitness for a particular use or any other expressed or implied warranty except as so stated above.

Polyguard-NSPW-Masterbatch

A polymeric intermediate antimicrobial additive engineered for integrated use in the manufacture of yarns, filaments, fibers, and knitted, woven or nonwoven textile fabrics, and subsequent manufactured treated article products.

Active Ingredient: Nanosilver*	1.00%
Other Ingredients:	99.00%
TOTAL:	100.00%

* Includes particles in the size range between 1 to 100 nm.

KEEP OUT OF REACH OF CHILDREN

CAUTION

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS

Causes Moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

WORKER PROTECTION

A long-sleeve shirt, long pants, shoes plus socks.

FIRST AID

IF IN EYES: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, the continue rinsing.

IF SWALLOWED: Rinse mouth and throat thoroughly with tap water, seek medical attention.

IF ON SKIN: Wash skin with soap and water, remove contaminated clothing.

ENVIRONMENTAL HAZARDS

This pesticide is toxic to fish, aquatic invertebrates, and birds. Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do Not discharge effluent containing this product to sewer systems without previously notifying the local sewer treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

EPA Registration No: 84610-E EPA Establishment No: 084610-FL-001
Net Contents: 25 kilograms

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

This product may not be used for applications involving food contact, food packaging, or drinking water.

Polyguard-NSPW Masterbatch is a polymeric Intermediate antimicrobial additive intended for commercial and industrial use. It is engineered for integrated use during the manufacturing process to impart durable antimicrobial activity to the manufactured product.

****See Technical Bulletin for detailed use information****

Polyguard-NSPW Masterbatch suppresses the growth of microbes which can cause unpleasant odors, discoloration, staining and deterioration of untreated manufactured products.

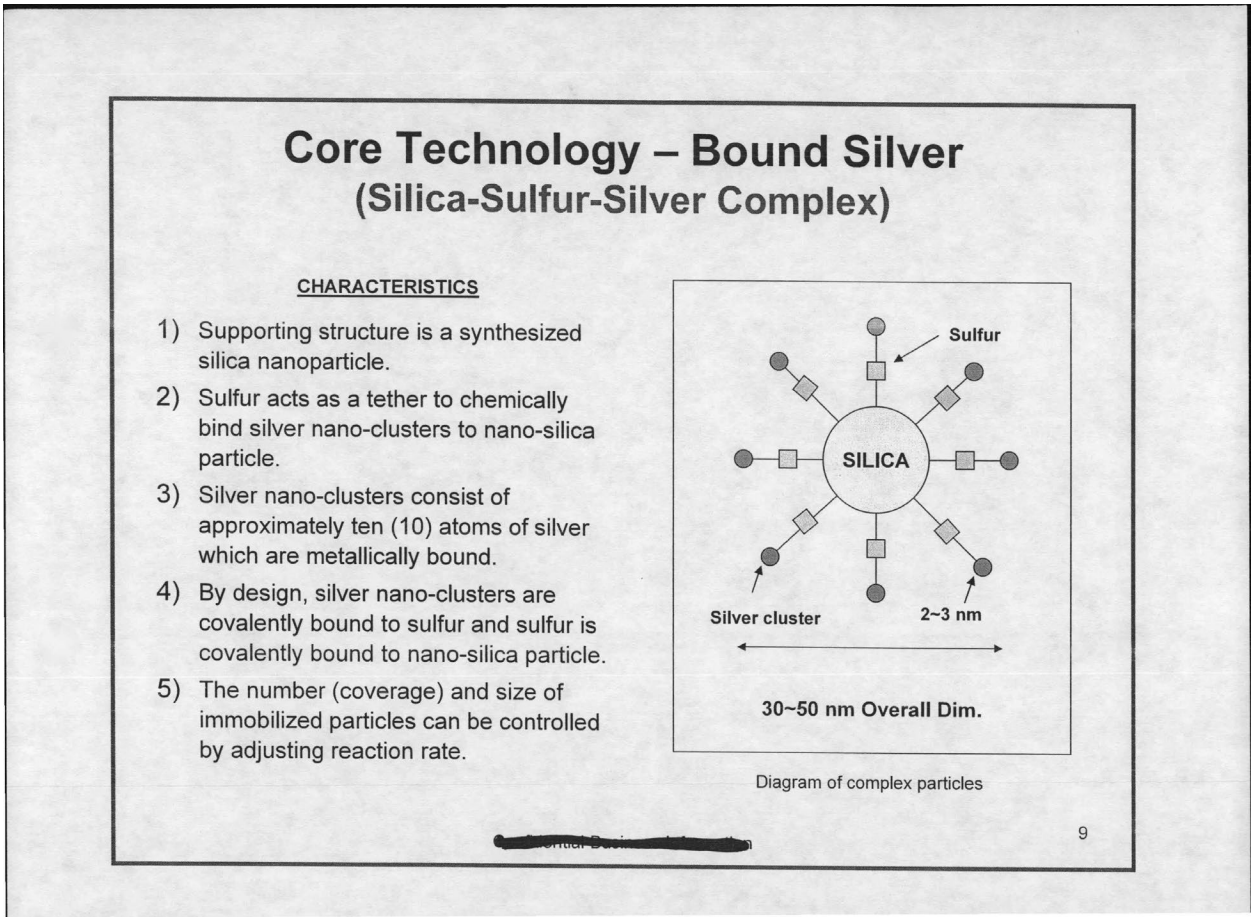
Finished products containing Polyguard-NSPW Masterbatch may not make public health claims relating to antimicrobial activity without EPA pesticide registration. When used in treated articles, this product does not protect users of any such treated article or others against food borne or disease causing bacteria, viruses or other disease causing organisms.

Polyguard-NSPW- Masterbatch is suitable for incorporation into treated articles listed in the technical bulletin. The Maximum application rate for treatment of finished product is 0.003% (by weight) or 30 ppm of nanosilver. Consult your Poly-Technical Solutions Ltd. sales engineer to determine the appropriate amount of Polyguard NSPW Masterbatch for your individual finished product.

DATE MANUFACTURED:
BATCH NUMBER:
LOT #:
EXPIRATION DATE:

APPENDIX B: NSPW Nanosilver[8] Particle Size and Appearance

Poly-Technical Solutions, Ltd. stated that the overall diameter of the NSPW Nanosilver particles is 30-50 nm, with the silver particles in the 2-3 nm range and dotting the surface of the silica core. The registrant presented a diagram[9] below that shows the appearance of the particles. Previous studies, MRID 49019201 (p. 7) and MRID 49045301 (p. 7), also provide the same description for the NSPW particles.



[8] Formerly referred to as NSPW-L30SS.
[9] Retrieved from page 9 of a PowerPoint titled "Nanosilva Antimicrobials: A New Standard In Performance and Protection", submitted in 2007.

APPENDIX C: Clarifications on NSPW-L30SS Morphology and Other Properties

The Agency has requested clarification from Poly-Technical Solutions regarding data and information submitted. The following is the Agency's current understanding of the answers received:

The NSPW-L30SS label lists silver concentration at 1.0%. This figure is calculated from the ratio of silver nitrate used as a reagent in the formulation of NSPW-L30SS, as shown in MRID 48652901. It does not represent a concentration of silver present in the final product. The concentration of silver present in the NSPW-L30SS solids is ~0.4% as shown in MRID 50617302, where ~0.3% is nanosilver and ~0.1% is silver ion. As the concentration of NSPW solids in solution is 0.2662 g/mL, the concentration of silver in the product is 0.0010648 g/mL, or 0.10648%, consisting of 0.07986% nanosilver and 0.02662% silver ions.

MRID 50617302 examined solids present in NSPW-L30SS. The relative concentration of silver found in MRID 50617302 (0.4%) does not match that provided in the elemental analysis from MRID 50699402 (1.8%). The Agency believes that this is due to a combination of natural batch-to-batch variation, as well as presence of water and other non-NSPW-L30SS ingredients and impurities in the dried sample in MRID 50617302; MRID 50617302 reports the silver concentration calculated from the total NSPW-L30SS solids, whereas MRID 50699402 reports the relative ratio of silver present in the nanoparticle.

NSPW-L30SS rapidly aggregates following synthesis, as was shown in MRID 50699402 (20-day-old samples). The material is immediately master batched into plastic pellets, which prevents aggregation from occurring. MRID 50649402 and MRID 50649401, which investigated the effect of temperature and duration on the morphology of the particles, measured samples (9 days old) with minimal aggregation and should be considered representative of the morphology of the material both at manufacture and for any potential consumer exposure. Further, EPA asked Wayne Krause from Poly-Technical Solutions, LTD., regarding the information in MRID 50649402: "What do the differing temperatures and durations signify? Do the differing temperatures and durations correlate to any specific conditions, such as shipping/handling or storage conditions, TEM-only conditions, incorporation of NSPW into batches?" Krause replied, "The effect of temperature and duration on morphology of particles was investigated. There was no significant (distinguishable) change in morphology."

The registrant provided a particle size frequency distribution table to clarify MRID 50649401. The NSPW particles were sonicated for 1 hour and measured by dynamic light scattering (DLS), and the distribution was number-based. The smallest percentile provided was 6.4%, and the particle size at that percentile was 28.21 nm. At the largest percentile (99.6%), the particle size was 190.1 nm. The average size was 43.67 nm.

In MRID 50617302, the concentration of solids found in the original solution was 0.2662 g/mL solution. This was used to dilute the original solution to a stock solution with the concentration of 1,000 mg/L for use in further measurements.

APPENDIX D: Ecotoxicity Data for Silver Ion

Terrestrial Animals

There is one acute avian oral study (MRID 46453301) on a high purity grade silver salt, silver chloride, in the inhouse database (Table D1). Silver chloride is classified as practically non-toxic to the Northern Bobwhite (*Colinus virginianus)* (LD50 > 2250 mg a.i./kg), expressed in terms of the amount of silver, the LD50 is >1,687 mg Ag/kg. An acute oral study with colloidal silver at a single dose showed no effects at 420 mg Ag/kg-bw (USEPA 1993).

Table D1: Silver Effects Data for Birds

% Purity	Endpoints as Silver, total [as test substance]	Toxicity Category of Test Substance	Study Classification/ Source/Comments
Silver chloride 99.6% (75% Ag)	15-d LD50 > 1687 mg Ag/kg [>2250 mg a.i./kg] 15-d NOAEL = 1012 mg Ag/kg [1350 mg a.i./kg]	Practically non-toxic	Acceptable/MRID 46453301

Aquatic Animals

The Agency used data contained within the USEPA AWQC silver documents (1980, 1987) for selecting endpoints. Data summarized in Eisler (1996) and Howe and Dobson (2002) were also considered, as in previous silver assessments. The following summarizes the data available in the 1987 AWQC public draft for silver (USEPA 1987). Acceptable data on acute effects of silver in freshwater was available for 12 species of invertebrates and 7 species of fish. Results in the 1987 public draft were not adjusted to a normalized hardness, whereas they were in the 1980 AWQC document. The public draft discusses issues associated with the hardness-dependent slope used to develop criteria in the 1980 AWQC, but the proposed silver criteria were not updated based on the 1987 approach. To be consistent with the current hardness-dependent criteria, with the exception of data from Goettl and Davies (1978), acute toxicity values were adjusted to a normalized hardness using the pooled slope of 1.72 from the 1980 AWQC document before selection of the most sensitive test and species. Hard water in Goettl and Davies (1978) tests was unusually toxic and therefore not used in setting the pooled slope. For selection of the most sensitive result for the risk assessment, results from Goettl and Davies (1978) were adjusted using the pooled slope of 0.341 from the three Goettl and Davies (1978) studies (0.098, 0.4815, 0.4444) to adjust their data for water hardness. There is also an additional public literature 96-hour LC50 of 1.9 ppb for the freshwater amphipod, *Hyallela azeteca,* (Howe and Dobson, 2002) which has been used in previous assessments of silver, because it was identified as the second most sensitive species as compared to cladocerans. Adjusting this value using the pooled slope

of 1.72 (USEPA 1980) results in adjusted values of 0.41, 3.5, and 70 ppb silver, total at water hardness of 15, 50, and 286 mg/L, respectively.

Excluding those values identified as outliers in the public draft, acute toxicity values normalized to a water hardness of 50 mg/L, ranged from 0.4 ppb for the cladoceran, *Daphnia magna*, to 3,402 ppb for the midge, *Tanytarsus dissimilis* (Table D2). There is chronic data for this species but all the ACRs are <1 (Table D3). These animals are not fed during acute testing but they are during chronic tests, and the presence of food appears to provide some protection from acute effects. This species was included in calculation of the silver AWQC and was therefore included in the risk assessment, but instead of using the lowest study value, the normalized Species Mean Acute Value (SMAV) of 1.08 ppb was used and the second most sensitive invertebrate included to allow determination of a reasonable chronic value for a sensitive invertebrate species that was not higher than the acute. The second most sensitive invertebrate species is a mayfly, *Leptophlebia* sp., with a normalized 96-hour LC_{50} of 2.5 ppb (Table D2).

The most sensitive freshwater fish test was with a fathead minnow, *Pimephales promelas*, with a normalized 96-hour LC_{50} of 2.5 ppb, the normalized fathead minnow SMAV is 9.5 ppb (Table D2). Two other fish species had lower normalized SMAVs, the speckled dace, *Rhinichthys osculus* (normalized SMAV = 6.8 ppb) and the mottled sculpin, *Cattus bairdi*, (normalized SMAV = 7.0 ppb), but no test result for these species were lower than that of the Fathead minnow (Table D2). To convert these values to dissolved metal the current national recommended conversion factor of 0.85 for silver acute studies was applied (Table D2).

Acceptable chronic toxicity data was available in the 1987 public draft, for a freshwater cladoceran, *D. magna*, two freshwater species of fish the Rainbow trout, *Oncorhynchus mykiss*, and the Fathead minnow *P. promelas,* and a saltwater invertebrate, the mysid *Americamysis bahia* (Table D3). Also considered in previous silver assessments is the chronic toxicity value for the freshwater amphipod *H. azteca* of 0.95 ppb (Howe and Dobson 2002). There is insufficient information for a chronic AWQC development and none currently exists.

As indicated previously a valid ACR for cladocerans cannot be determined because the ACRs are <1 (Table D3). The ACR for *H. azteca,* a species of similar acute sensitivity as the cladoceran and mayfly is 2.0 (1.9/0.95 = 2). This differs by about a factor of 10 from the 1987 FACR adjusted to a NOEC basis of 21.39 (Table D3). The *H. azteca* ARC of 2.0 was used for the acutely sensitive mayfly. The 1987 FACR, adjusted for use of a NOEC, of 21.39 (Table D3) was used to estimate a chronic value for the fathead minnow.

Table D2: Excerpt of Acute Effects Data for the Most Sensitive Invertebrate and Fish Species from the USEPA 1987 Silver Criteria Documents Showing Values Adjusted to Water Hardness of 15, 50, and 286 mg/L as $CaCO_3$

Test Species	Exposure Method	AWQC Pooled Slope[a]	Actual Study Hardness	Study Toxicity Value (ppb)	Ln(h1)	Ln(t1)	Adjusted Toxicity Value (ppb), as Total Recoverable, at Water Hardness[d]:			Adjusted Toxicity Value (ppb), as Dissolved[e], at Water Hardness:			SMAV
		m	h1	t1	x1	y1	15	50	286	15	50	286	
AWQC CMC[f]	--	1.72	50	1.23	3.9120	0.2070	0.155	1.23	24.7	0.132	1.05	21.0	-
Cladoceran, *Daphnia magna*	S, M	1.72	54	2.2	3.9890	0.7885	0.24	1.9	39	0.21	1.6	33	1.08
Cladoceran, *Daphnia magna*	S, M	1.72		1.07									
Cladoceran, *Daphnia magna*	S, M	1.72		0.64									
Cladoceran, *Daphnia magna*	S, U	1.72		0.39									
Cladoceran, *Daphnia magna*	S, M	1.72	255	48	5.5413	3.8712	0.37	2.9	58	0.31	2.5	50	
Cladoceran, *Daphnia magna*	S, M	1.72	255	55	5.5413	4.0073	0.42	3.3	67	0.36	2.8	57	
Cladoceran, *Daphnia magna*	S, M	1.72	73	8.4	4.2905	2.1282	0.55	4.4	88	0.47	3.7	75	
Cladoceran, *Daphnia magna*	S, M	1.72	73	14.9	4.2905	2.7014	0.98	7.8	156	0.83	6.6	133	
Cladoceran, *Daphnia magna*	S, M	1.72	60	1.1	4.0943	0.0953	0.10	0.8	16	0.09	0.68	13.7	
Cladoceran, *Daphnia magna*	S, M	1.72	60	0.6	4.0943	-0.5108	0.06	0.4	9	0.05	0.37	7.5	
Cladoceran, *Daphnia magna*	S, M	1.72	46	0.63	3.8286	-0.4620	0.09	0.73	15	0.078	0.6	12	
Cladoceran, *Daphnia magna*	S, M	1.72	46	0.66	3.8286	-0.4155	0.10	0.76	15	0.082	0.6	13	
Cladoceran, *Daphnia magna*	S, M	1.72	46	0.9	3.8286	-0.1054	0.13	1.0	21	0.11	0.9	18	
Cladoceran, *Daphnia magna*	S, M	1.72	46	1.03	3.8286	0.0296	0.15	1.19	23.9	0.127	1.01	20.3	
Cladoceran, *Daphnia magna*	S, M	1.72	54	2.9	3.9890	1.0647	0.32	2.5	51	0.27	2.2	43	
Cladoceran, *Daphnia magna*	S, M	1.72	47	0.24	3.8501	-1.4271	0.03	0.27	5.4	0.029	0.23	4.6	(f)
Cladoceran, *Daphnia magna*	S, M	1.72	60	1.1	4.0943	0.0953	0.10	0.80	16	0.09	0.68	14	
Cladoceran, *Daphnia magna*	S, M	1.72	39	0.6	3.6636	-0.5108	0.12	0.92	18	0.10	0.78	16	
Cladoceran, *Daphnia magna*	S, U	1.72	72	1.5	4.2767	0.4055	0.10	0.80	16	0.09	0.68	14	
Cladoceran, *Daphnia magna*	S, U	1.72	240	10	5.4806	2.3026	0.08	0.67	14	0.07	0.6	11	
Cladoceran, *Daphnia magna*	S, U	1.72	240	1.5	5.4806	0.4055	0.01	0.10	2.0	0.01	0.1	2	(f)
Cladoceran, *Daphnia magna*	F, M	1.72	44.7	0.9	3.8000	-0.1054	0.14	1.1	21.9	0.12	0.9	19	
Cladoceran, *Daphnia pulex*	S, U	1.72	45	14	3.8067	2.6391	2.12	17	337	1.8	14.3	286	
Cladoceran, *Daphnia pulex*	S, U	1.72	240	1.9	5.4806	0.6419	0.016	0.1	2.57	0.014	0.109	2.18	(f)
Mayfly, *Leptophlabia* sp.	S, M	1.72	46.6	2.2	3.8416	0.7885	0.313	2.48	49.9	0.266	2.111	42.4	2.48

Test Species	Exposure Method	AWQC Pooled Slope[a]	Actual Study Hardness	Study Toxicity Value (ppb)	Ln(h1)	Ln(t1)	Adjusted Toxicity Value (ppb), as Total Recoverable, at Water Hardness[d]:			Adjusted Toxicity Value (ppb), as Dissolved[e], at Water Hardness:			SMAV
		m	h1	t1	x1	y1	15	50	286	15	50	286	
Fathead minnow, *Pimephales promelas*	S, M	1.72	48	30.43	3.8712	3.4154	4.115	32.6	655	3.50	27.7	557	9.5
Fathead minnow, *Pimephales promelas*	S, M	1.72	255	230	5.5413	5.4381	1.759	14.0	280	1.50	11.9	238	
Fathead minnow, *Pimephales promelas*	S, M	1.72	54	13.8	3.9890	2.6247	1.524	12.1	243	1.30	10.3	206	
Fathead minnow, *Pimephales promelas*	S, M	1.72	46.1	6.7	3.8308	1.9021	0.971	7.7	155	0.826	6.55	131	
Fathead minnow, *Pimephales promelas*	S, M	1.72	75	10.3	4.3175	2.3321	0.647	5.1	103	0.550	4.36	87.5	
Fathead minnow, *Pimephales promelas*	S, M	1.72	48	22.66	3.8712	3.1206	3.06	24	488	2.60	20.7	415	
Fathead minnow, *Pimephales promelas*	S, M	1.72	255	270	5.5413	5.5984	2.07	16.4	329	1.76	13.9	280	
Fathead minnow, *Pimephales promelas*	S, M	1.72	54	19.6	3.9890	2.9755	2.16	17	345	1.84	14.6	293	
Fathead minnow, *Pimephales promelas*	S, M	1.72	46.1	12.3	3.8308	2.5096	1.78	14.1	284	1.52	12.0	241	
Fathead minnow, *Pimephales promelas*	S, M	1.72	75	8.7	4.3175	2.1633	0.546	4.3	87.0	0.464	3.68	73.9	
Fathead minnow, *Pimephales promelas*	F, M	1.72	40	5.6	3.6889	1.7228	1.04	8.2	165	0.881	6.99	140	
Fathead minnow, *Pimephales promelas*	F, M	1.72	36	7.4	3.5835	2.0015	1.64	13.0	261	1.40	11.1	222	
Fathead minnow, *Pimephales promelas*	S, M	1.72	38	9.4	3.6376	2.2407	1.90	15.1	303	1.61	12.8	257	
Fathead minnow, *Pimephales promelas*	S, M	1.72	39	9.7	3.6636	2.2721	1.87	14.9	299	1.59	12.6	254	
Fathead minnow, *Pimephales promelas*	S, M	1.72	44.8	14	3.8022	2.6391	2.13	16.9	340	1.81	14.4	289	
Fathead minnow, *Pimephales promelas*	F, U	0.341	33	3.9	3.4965	1.3610	2.98	4.5	8.1	2.5	3.8	6.9	
Fathead minnow, *Pimephales promelas*	F, U	0.341	274	4.8	5.6131	1.5686	1.782	2.7	4.9	1.5	2.3	4.1	
Fathead minnow, *Pimephales promelas*	F, M	1.72	44.7	9	3.8000	2.1972	1.38	10.9	219	1.17	9.28	186	
Fathead minnow, *Pimephales promelas*	F, M	1.72	38	16	3.6376	2.7726	3.23	25.7	515	2.75	21.8	438	

Test Species	Exposure Method	AWQC Pooled Slope[a]	Actual Study Hardness	Study Toxicity Value (ppb)	Ln(h1)	Ln(t1)	Adjusted Toxicity Value (ppb), as Total Recoverable, at Water Hardness[d]:			Adjusted Toxicity Value (ppb), as Dissolved[e], at Water Hardness:			SMAV
		m	h1	t1	x1	y1	15	50	286	15	50	286	
Fathead minnow, *Pimephales promelas*	F, M	1.72	46	10.7	3.8286	2.3702	1.56	12.3	248	1.32	10.5	211	
Fathead minnow, *Pimephales promelas*	F, M	1.72	48	10.98	3.8712	2.3961	1.48	11.8	236	1.26	10.0	201	
Fathead minnow, *Pimephales promelas*	F, M	1.72	255	150	5.5413	5.0106	1.15	9.1	183	0.975	7.74	155	
Fathead minnow, *Pimephales promelas*	F, M	1.72	54	11.1	3.9890	2.4069	1.23	9.7	195	1.042	8.26	166	
Fathead minnow, *Pimephales promelas*	F, M	1.72	46.1	5.3	3.8308	1.6677	0.768	6.1	122	0.653	5.18	104	
Fathead minnow, *Pimephales promelas*	F, M	1.72	75	6.3	4.3175	1.8405	0.395	3.1	63.0	0.336	2.67	53.5	
Fathead minnow, *Pimephales promelas*	F, M	1.72	48	11.75	3.8712	2.4639	1.59	12.6	253	1.351	10.7	215	
Fathead minnow, *Pimephales promelas*	F, M	1.72	255	110	5.5413	4.7005	0.841	6.7	134	0.715	5.67	114	
Fathead minnow, *Pimephales promelas*	F, M	1.72	46.1	3.9	3.8308	1.3610	0.565	4.5	90.0	0.481	3.8	76.5	
Fathead minnow, *Pimephales promelas*	F, M	1.72	75	5	4.3175	1.6094	0.314	2.5	50.0	0.267	2.1	42.5	
Fathead minnow, *Pimephales promelas*	F, M	1.72	44.4	6.7	3.7932	1.9021	1.04	8.2	165	0.881	6.99	140	
Speckled dace, *Rhinichthys osculus*	F, U	0.341	30	4.9	3.4012	1.5892	3.9	5.8	11	3.3	5.0	9	6.77
Speckled dace, *Rhinichthys osculus*	F, U	0.341	250	13.6	5.5215	2.6101	5.2	7.9	14.2	4.4	6.7	12.1	
Mottled sculpin, *Cattus bairdi*	F, U	0.341	30	5.3	3.4012	1.6677	4.2	6.3	11	3.6	5.4	10	7.04
Mottled sculpin, *Cattus bairdi*	F, U	0.341	250	13.6	5.5215	2.6101	5.2	7.9	14.2	4.4	6.7	12.1	

(a) Except for the final mean acute value in the first row, values are from Table 1 in USEPA 1987 adjusted to hardness of 50 mg/L as $CaCO_3$ using, except where noted, the pooled slope of 1.72 from USEPA 1980. Hard water in Goettl and Davies (1978) tests was unusually toxic and therefore not used in setting the pooled slope. For selection of the most sensitive result for the risk assessment, results from Goettl and Davies (1978) were adjusted using the pooled slope of 0.341 from the Goettl and Davies (1978) studies (0.098, 0.4815, 0.4444) were used for adjustments for these studies.

(b) Except where noted in table footnote (a), the acute slope is from Appendix B of current Recommended National Ambient WQC

(c) Natural log values of water hardness (x = Ln(hardness)) at 15, 76, 136, and 286 mg CaCO3/L are 2.7080, 3.9120, 4.3307, 4.9126, and 5.6560, respectively.

(c) Adjusted toxicity value (y) = $m(x - x_1) + y_1$ from point-slope linear relationship; see table footnote (c) for definition of x.

(d) Exp(y); see table footnote (b) for definition of y.

(e) The current Final Acute Value from Appendix B of current Recommended National Ambient WQC given as exp(1.72*[ln(hardness)], based on USEPA 1980. http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm

(f) Values from Elnabarawy *et al*. 1986 were excluded from SMAV calculations. Results from the hard water used in this laboratory for this species and others in the USEPA 1987 appear to be more toxic than hard water at other laboratories for the same species, and values tend to be greater than a factor of 10 from other adjusted values. These studies are shaded in orange.

Table D3: Silver AWQC ACRs Adjusted to NOEC Basis

	Hardness (mg/L as CaCO3)	Acute value (ppb)	NOEC (ppb)	LOEC (ppb)	Chronic Value (ppb) = MATC (a)	ACR based on MATC	SMACR used to calculate FMACR for AWQC	ACR based on NOEC	SMACR Adjusted to NOEC	Reference
Cladoceran, *Daphnia magna*	73	11.2 (b)	10.5	21.2	14.92	0.7507	0.5015	1.067	0.743	Nebeker 1982
Cladoceran, *Daphnia magna*	73	11.2 (b)	20.0	41.0	28.64	0.3911		0.560		Nebeker 1982
Cladoceran, *Daphnia magna*	60	1.1	1.6	4.1	2.561	0.4295		0.688		Nebeker *et al*. 1983; Nebeker 1982
Rainbow trout, *Oncorhynchus mykiss*	36	9.2	0.36	0.51	0.4285	21.47	33.29	25.56	42.63	Nebeker *et al*. 1983
Rainbow trout, *Oncorhynchus mykiss*	28	6.4	0.09	0.17	0.124	51.61		71.11		Davies *et al*. 1978
Fathead minnow, *Pimephales promelas*	44.8	6.7	0.37	0.65	0.4904	13.66	13.66	18.11	18.11	Holcombe *et al*. 1983
Mysid, *Americamysis bahia*	30	249	11	32	18.76	13.2729	8.51	22.636	12.68	McKenny 1982; Lussier *et al* 1985
Mysid, Americamysis *bahia*	15-30	86	14	19	16.31	5.2728		6.143		McKenny 1982
Mysid, *Americamysis bahia*	15-30	132	9	25	15.00	8.8000		14.667		McKenny 1982
Final mean acute-to-chronic ratio (FMACR)							15.70(c)		21.39	

(a) Maximum acute threshold concentration (MATC) which is the geometric mean of the NOEC and LOEC values.
(b) Geometric mean of the two acute tests conducted at this laboratory, under same water hardness conditions (8.4 and 14.9 ppb).
(c) SMACR for the daphnids was not included in the FMACR calculations. ACRs should be greater than 1, and as explained in the USEPA (1980, and 1987) silver water quality criteria documents the presence of food in the chronic tests with the cladocerans appears to make the organisms less sensitive.

Aquatic Plants

Summarized in Table D4 are the most sensitive aquatic plant endpoints used in previous assessment for silver. These values are based on silver data within OPP's files and selected open literature: EPA (1987) draft ambient aquatic life criteria document; Eisler (1996) synoptic review of silver hazards to fish, wildlife, and invertebrates; Howe and Dobson (2002) World Health Organization synoptic review of silver and silver compound fate and effects.

Table D4: Summary of Silver Toxicity to Aquatic Plants

Plant	Toxicity Value	Source
Freshwater green alga, *Selenastrum capricornutum*	4-day IC50 = 2.6 ppb (chlorophyll *a*)	USEPA 1987
Saltwater dinoflagellate, *Prorocentrum mariaelebouriae*	5-day IC50 = 3.3 ppb (7.5 ppt salinity, growth)	Eisler 1996; Howe and Dobson, 2002
Saltwater diatom, *Skeletonema costatum*	5-day IC50 = 5.9 ppb (7.5 ppt salinity, growth)	Eisler, 1996; Howe and Dobson, 2002
Red alga, *Champia parvula*)	28-d NOAEC = 1.2 ppb (cystocarp formation)	EPA, 1987
Blue-green *Micryocystis aeruginosa* and *Cylindrosperum licheniforme*	IC50 = 420 ppb	EPA, 1980
Duckweed, *Lemna minor*	28-d IC50 = 270 ppb	EPA, 1987/ (Brown and Rattigan, 1979)
Terrestrial Plant – lettuce (germination), *Lactuca sativa*	>750 ppb	Howe and Dobson, 2002

APPENDIX E: NSPW Nanosilver 158(w) Data Requirements Table

158.2210 Product Chemistry			
Guideline Number	Data Requirement	Use Pattern	Notes:
830.1550	Product identity and composition	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828901, -03, -05, 50649402
830.1600	Description of materials used to produce the product	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828902 (Rodriquez's memo incorrectly cites -01)
830.1620	Description of production process	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828903
830.1650	Description of formulation process	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828904
830.1670	Description of formulation of impurities	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828905
830.1700	Preliminary analysis	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828906
830.1750	Certified limits	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828907
830.1800	Enforcement analytical method	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828908
830.1900	Submittal of samples	CR	Addressed in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. "Samples are to be provided on a case-by-case basis for end-use products"
830.6302	Color	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRIDs 50649402, 47828902, 47828909
830.6303	Physical state	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRIDs 50649402, 47828910, 47828917

830.6304	Odor	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828911
830.6313	Stability to normal and elevated temperatures, metals, and metal ions	R	Declared "NR" in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885 Relevant MRID 47828912 for room temperatures and corrosion. Room temperature aging and elevated temperature assessed in MRID 50649402 (100°C for 6 hours). Additional information regarding these data is discussed in Appendix C.
830.6314	Oxidation/reduction: chemical incompatibility	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828902
830.6315	Flammability	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828902
830.6316	Explodability	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828902
830.6317	Storage stability	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828912
830.6319	Miscibility	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015. Relevant MRID 47828902
830.6320	Corrosion characteristics	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828912
830.6321	Dielectric breakdown voltage	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828902
830.7000	pH	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRIDs 47828902, 47828913
830.7050	UV/Visible light absorption	R	Declared "NR" in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. However, data are supplied in other studies: (MRID 50617302, the Georgia Tech characterization study for the

			concentration, and MRID 50649402, the TEM data for particle size) can be considered acceptable and fulfills the UV/VIS data requirement.
830.7100	Viscosity	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828914
830.7200	Melting point/melting range	R	Not required. Per test note 20, only required if proposed pesticide product is a solid. Here, NSPW is a liquid suspension.
830.7220	Boiling point/boiling range	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828915 (Rodriquez's memo incorrectly cites -14)
830.7300	Density/relative density/bulk density	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRIDs 47828902, 47828916
830.7370	Dissociation constants in water	R	Declared "NR" in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. No explanation. MRID 50617302 provides acceptable information on the dissolution kinetics.
830.7520	Particle size, fiber length, and diameter distribution	CR	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRIDs 47828901, -02, -03, -04, 50699402, 50617302, and 50649402
830.7550 830.7560 830.7570	Partition coefficient (n-octanol/water)	R	Per test note 24, not pertinent to inorganic molecules
830.7840 830.7860	Water solubility	R	Accepted in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Relevant MRID 47828917
830.7950	Vapor pressure	R	Declared "NR" in memo "Review for 84610-E" S. Rodriguez, 5/12/2015, DP 402885. Data are not required because the metal complexes are not expected to volatilize.

Note: The studies used to fulfill the guideline requirements above also satisfy the special non-guideline requirements called for in the 2018 Nanosilver FWP. The non-guideline requirements are:

- Particle Size and Diameter (Size) Distribution – satisfied with DLS data in MRIDs 50699402, 50617302, and 50649401, and TEM data in MRIDs 50649402 and 50699402. The DLS and TEM data were sufficient that SEM data were no longer needed.
- Surface Area Determination – satisfied with MRID 50699402
- Zeta Potential – satisfied with MRID 50699402

- Stability to Sunlight, Detergents, Temperature, and Salinity – not required. See notes for GLN 830.6313 regarding temperature. Based on textile leaching study (MRID 49190801), very low amounts of silver were released during laundry wash, suggesting low potential exposure; in addition, silver is in a metallic state and stabilized with a stabilizing agent.
- Rate of Deposition/Aggregation – not required because of low potential exposure in the aquatic environment, and some information on dissolution has already been provided by MRID 50617302
- Dissolution Kinetics – satisfied with MRID 50617302

Other special non-guideline requirements in the 2018 Nanosilver FWP that are waived:

- Plastic Leaching Study – not required because plastic use has been removed
- Peri- and Postnatal Exposure to NSPW-Nanosilver – not required because plastic use has been removed

158.2230 Toxicology			
Guideline No.	Data Requirement	Use Pattern- Textile, non-food use	Notes:
870.1100	Acute oral toxicity- rat	R	Acceptable data submitted. MRID 47828918.
870.1200	Acute dermal toxicity	R	Acceptable data submitted. MRID 47828919.
870.1300	Acute inhalation toxicity- rat	R	Acceptable data submitted. MRID 47828920.
870.2400	Primary eye irritation- rabbit	R	Acceptable data submitted. MRID 47828921.
870.2500	Primary dermal irritation	R	Acceptable data submitted. MRID 47828922.
870.2600	Dermal sensitization	R	Acceptable data submitted. MRID 47828923.
870.2600	Acute neurotoxicity- rat	CR	Previously waived based on closed system loading and related neurotoxicity data. For non-food uses, data are required if oral studies show neurotoxic effects. In August 2013, HasPoc recommended that neurotoxicity of the nanosilver in the product be evaluated in a subchronic inhalation study. This recommendation was based on potential neurotoxic effects seen after administration of nasal drops. No neurotoxic effects were seen after intraperitoneal

			injection and the effects seen in one oral study were not seen in follow-up oral studies.. The subchronic inhalation study is now not required because there are no inhalation exposures expected, *e.g.*, due to masterbatch formulation
870.3100	90-Day oral toxicity- rat	CR	Waived because a NOAEL in the subchronic oral toxicity studies found in open literature on nanosilvers can be used to establish a POD for assessing risks for the nanosilver in NSPW. Since oral exposures are low and incidental oral MOEs are high, additional data would not change the risk analysis.
870.3150	90-Day oral toxicity- nonrodent	CR	Not required because active ingredient is not bioaccumulative. Further, since oral exposures are low and incidental oral MOEs are high, additional data would not change the risk analysis
870.3200	21/28-Day dermal toxicity	CR	Waived because endpoints for risk assessment based on an oral endpoint and a dermal absorption factor of 6.7 percent. HasPoc held in August, 2013. .
870.3250	90-Day dermal toxicity	CR	
870.3465	90-Day inhalation toxicity- rat	CR	Not required because the proposed product is a masterbatch therefore, there is no inhalation exposure.
870.6200	90-Day neurotoxicity- rat	CR	Waived. In August 2013, HasPoc recommended that neurotoxicity to workers? of the nanosilver in the product be evaluated in a subchronic inhalation study. This recommendation was based on potential neurotoxic effects seen after administration of nasal drops and assuming inhalation exposure. No neurotoxic effects were seen after intraperitoneal injection and the effects seen in one oral study were not seen in follow-up oral studies. The subchronic inhalation study is now not required because there are no inhalation exposures expected, *e.g.*, due to masterbatch.
870.4100	Chronic oral toxicity- rodent	CR	Not required because incidental oral exposures are not chronic. Significant human exposure over a considerable portion of the human lifespan (which is significant in terms of frequency, time, duration, and/or magnitude of exposure) is not expected.[1] https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf
870.4200	Carcinogenicity- two rodent species- rat and mouse preferred	CR	

870.3700	Prenatal developmental toxicity- rat and rabbit preferred	R	Waived based on use of data in the open literature for nanosilvers generally, allowing conclusion that PODs are protective since oral exposures are low and incidental oral MOEs are high, and no inhalation exposures are expected.
870.3800	Reproduction and fertility effects	R	Waived. In August 2013, HasPoc recommended that potential reproduction effects of the nanosilver in the product be evaluated in a subchronic inhalation study. The subchronic inhalation study is now not required because there are no inhalation exposures expected, *e.g.*, due to masterbatch.
870.6300	Developmental neurotoxicity	CR	Not required since no Weight of Evidence that nanosilvers cause neurological effects in developing animals.
870.5100	Reverse mutation assay	R	Waived because the Agency has reviewed open literature on nanosilvers and these studies are adequate to elucidate the mutagenic potential of the chemical. Even though there are differences in nanosilvers in the open literature and nanosilver in NSPW, requiring mutagenesis studies on this product will not yield data that would update this risk assessment.
870.5300 870.5375	In vitro mammalian gene mutation	R	
870.5385 870.5395	In vivo cytogenetics	R	
870.7485	Metabolism and pharmacokinetics	CR	Not required because chronic and carcinogenicity studies are not required.
870.7200	Companion animal safety	CR	Not required. Not used on companion animals.
870.7600	Dermal penetration	CR	Waived. Used dermal adsorption factor from open literature.
870.7800	Immunotoxicity	R	Waived by HasPoc in August, 2013.

1: https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

Note:

158.2240: Nontarget Organisms and 158.2250: Nontarget Plant Protection			
Guideline No	Study	Use Pattern- "All other use patterns category"	Notes:
850.2100	Acute Avian Oral Tox	R[2]	Utilized for labeling purposes. Waived in Appendix A of the 2015 NSPW-L30SS Decision Memo.

			Study is not required based on the results of the current risk assessment utilizing the most sensitive species and the current application rate. Cautionary labeling language is recommended.[1]
850.1010	Acute Freshwater Invertebrate Tox	R	Acceptable data. MRID 50617301, 50699401 (updated)
850.1075	Acute Freshwater Fish Tox	R[2]	Utilized for labeling purposes. Waived in Appendix A of the 2015 NSPW-L30SS Decision Memo. Study is not required based on the results of the current risk assessment utilizing the most sensitive species and the current application rate. Cautionary labeling language is recommended.[1]
850.2200	Avian Dietary Tox	CR	Not required because terrestrial exposure to nanosilver not expected.
850.2300	Avian Reproduction	CR	Not required because birds are not expected to be subject to repeated or continued exposure to nanosilver in the textiles.
850.1025	Acute Estuarine and Marine organisms tox	CR	Not required because no marine exposure to nanosilver expected
850.1035, 850.1045, 850.1055	Acute Estuarine and Marine Organisms Tox	CR	
850.1075, 850.1400	Fish Early-Life Stage	R	Required, but waived because no chronic exposure to nanosilver expected
850.1300	Aquatic Invertebrate Life cycle	R	
850.1350, 850.1500,	Fish Life Cycle	CR	Not required because no chronic exposure to nanosilver expected
850.1710	Aquatic Organism, Bioavailability, Biomagnification, and Toxicity Tests	CR	Not required because no biomagnification expected based on low aquatic exposure to nanosilver.
850.1730, 850.1850, 850.1950	Simulated or Actual Field Testing for Aquatic Organisms	CR	Not required because significant potential exposure to nanosilver is not expected.
850.1735	Whole Sediment; Acute Freshwater Invertebrates	CR	Not required because no sediment exposure to nanosilver expected.
850.1740	Whole sediment; Acute Marine Invertebrates	CR	

None	Whole Sediment; Chronic Invertebrate Freshwater and Marine	CR	Not required because no chronic exposure to nanosilver expected
850.3020	Honeybee Acute Contact	CR	Not required because no terrestrial exposure to nanosilver expected
850.3030	Toxicity of Residues to Honeybees	CR	
850.4225	Seedling Emergence, Tier II- Dose Response	CR	Not required because no semi-aquatic exposure to nanosilver expected
850.4250	Vegetative Vigor Tier II- Dose Response	CR	
850.4400	Aquatic Plant Growth (Aquatic Vascular Plant) Tier II- Dose Response	CR	Not required based on results of the screening level risk-assessment utilizing the most sensitive species and the current application rate.
850.4500/ .4550	Aquatic Plant Growth (algal) Tier II (dose Response)	R[2]	Not required during the 2015 registration. Study is not required based on the results of the current risk assessment utilizing the most sensitive species and the current application rate. Cautionary labeling language is recommended.[1]
850.4300	Terrestrial Field	CR	Not required because no exposure to nanosilver expected in terrestrial fields.
850.4450	Aquatic Field	CR	Not required because no exposure to nanosilver expected in aquatic fields.

1: The current assessment evaluated the acute risk to the species most sensitive to other types of silver (silver ions, nanosilver from other sources, *etc.*) and found no risks from nanosilver derived from NSPW Nanosilver. Therefore, the risk conclusions based on the *Daphnia* data are assumed to be protective of other aquatic organisms (including aquatic plants) and no additional ecotoxicity data are required at this time to support the NSPW Nanosilver registration for use as a material preservative in textiles. No additional ecotoxicity data have been received. Based on the toxicity in the daphnid study, the Agency will require cautionary language for non-target species on the label.

2: Four studies are generally required for labeling purposes. The 2009 waiver request: MRID 47828924 asked for waivers for the avian and fish studies. This waiver was accepted in Appendix A of the 2015 Registration Decision for NSPW Nanosilver. These data are not needed for risk assessment and in the absence of the data, cautionary language is often added to the label.

158.2260: Applicator Exposure			
Guideline No	Study	Use Site-Occupational	Notes:
875.1100/.1200	Dermal Exposure	R	Waived or not required because the product is a masterbatch.
875.1300/.1400	Inhalation Exposure	R	
875.1500	Biological Monitoring	CR	

875.1600	Data Reporting and Calculations	R	
875.1700	Product Use Information	R	

158.2270: Post-Application Exposure			
Guideline No	Study	Use Site-Occupational	Notes:
875.2200	Soil Residue Dissipation	CR	Not required because not relevant for textiles.
875.2300	Indoor Surface Residue Dissipation	CR	Acceptable data. MRID 49190801
875.2400	Dermal Exposure	CR	Acceptable data. MRID 49190801
875.2500	Inhalation Exposure	CR	Not required because not relevant for textiles.
875.2600	Biological Monitoring	CR	Not required because this is an optional study that can be used to address 875.2400 or 875.2500.
875.2700	Product Use Information	R	Waived. These data are generally used to provide information about historical use of the product. Not required for new active ingredients.
875.2800	Description of Human Activity	R	
875.2900	Data Reporting and Calculations	R	Not currently required as no related studies or parameters are currently needed for review.

158.2280: Environmental Fate			
Guideline No	Study	Use Pattern-"All other use patterns category"	Notes:
835.2120	Hydrolysis	R	Waived because the study is not pertinent to inorganic molecules.
835.2240	Photodegradation, in Water	R	Waived because the study is not pertinent to inorganic molecules.
835.2410	Photodegradation, in Soil	R	Waived because the study is not pertinent to inorganic molecules.
850.6800	Activated Sludge, Respiration Inhibition Test	NR	Not required because conservative assumptions in the DtD assessment resulted in no risks. If the DtD assessment had resulted in risks, these data would refine the assessment.
835.1110	Activated Sludge Sorption Isotherm	R	
835.3110	Ready Biodegradability	CR	
835.3200	Porous Pot Study	CR	
835.3280	Simulation Tests to Assess the	CR	

	Biodegradability of Chemicals in Wastewater		
835.3240	Simulation Test- Aerobic Sewage Treatment: A. Activated Sludge Units	CR	
835.1230	Leaching and Adsorption/Desorption	CR	Waived because there would be minimal soil exposure.
835.4100	Aerobic Soil Metabolisms	CR	Waived because the study is not pertinent to inorganic molecules.
835.4200	Anaerobic Soil Metabolism	CR	Waived because the study is not pertinent to inorganic molecules.
835.4300	Aerobic Aquatic Metabolisms	CR	Waived because the study is not pertinent to inorganic molecules.
835.4400	Anaerobic Aquatic Metabolism	CR	Waived because the study is not pertinent to inorganic molecules.
835.6200	Aquatic (sediment)	CR	Waived because the study is not pertinent to inorganic molecules.
None	Monitoring of Representative US Waters	CR	Not required based on low potential exposure.
None	Special Leaching	NR	Acceptable data. MRID 49019201

158.2290: Residue Chemistry- Not required because the product has non-food uses.

APPENDIX F: Ecotoxicity Open Literature References Screened

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Ribeiro, F., Gallego-Urrea, J. A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., ... & Loureiro, S. (2014). Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. *Science of the Total Environment*, *466*, 232-241.

Stevenson, L. M. (2016). *Ecological feedbacks and engineered nanomaterials in freshwater environments*(Doctoral dissertation, UC Santa Barbara).

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Walters, C. R., Pool, E. J., & Somerset, V. S. (2014). Ecotoxicity of silver nanomaterials in the aquatic environment: a review of literature and gaps in nano-toxicological research. *Journal of Environmental Science and Health, Part A*, *49*(13), 1588-1601.

Zhang, X. (2017). *Ecotoxicological Effects of Silver Nanoparticles: The Relevance of Test Species and Test Conditions* (Doctoral dissertation, Universität Bremen).

APPENDIX G: Summaries of Toxicological Studies Cited in the 2015 Registration Decision for NSPW-L30SS.

Acute Toxicology Studies Available for Analysis

The registrant submitted results from guideline acute animal-toxicity tests completed using high-level doses of a liquid suspension containing NSPW-L30SS. As outlined in Table 10, there were no mortalities or abnormalities noted in test animals after administration of NSPW-L30SS by oral, dermal, or inhalation routes at dose levels of up to 5,000 mg/kg and 2.07 mg/L, (gravimetric; nominal = 215.62 mg/L; mean aerodynamic diameter = 2.5 μm) respectively; NSPW-L30SS caused moderate to no irritation to skin or eyes at dose levels of up to 0.1 mL and 0.5 mL, respectively; and was not a skin sensitizer. According to EPA's Toxicity Category system, which is used for product labeling purposes, shipping containers filled with NSPW-L30SS are required to carry a label stating "CAUTION" where contact with eyes or clothing should be avoided.

Table 10: Acute Toxicity Profile for the NSPW-L30SS Liquid Suspension

Study	Result	Toxicity Category
Acute Oral Toxicity	No mortality or abnormalities after dose of 5,000 mg/kg	IV
Acute Dermal Toxicity	No mortality or abnormalities after dose of 5,000 mg/kg	IV
Acute Inhalation Toxicity	No mortality or abnormalities after dose of 2.05 mg/L	IV
Acute Eye Irritation	Moderate to not irritating after dose of 0.1 mL	III
Acute Dermal Irritation	Mild or slight irritation after dose of 0.5 mL	IV
Skin Sensitization	Not a sensitizer	N/A

Repeat Dose Toxicology Studies Available for Analysis

There are no repeated-dose subchronic or chronic toxicity studies available for NSPW-L30SS or the nanosilver in NSPW-L30SS. However, there are repeated-dose toxicity studies available in the scientific literature for nanosilver. The human health toxicological data requirements and the basis for waiving these data are listed in Appendix E. Since nanosilver might be released from NSPW-L30SS and articles incorporating NSPW-L30SS, EPA considers the scientific literature studies on nanosilver toxicity relevant. EPA believes the studies described in the following sections are sufficient for assessing the risks from the use of NSPW-L30SS. The database for nanosilver is sufficient because the studies encompasses various routes of exposure, the methods measure the necessary endpoints the agency uses for risk assessment, and the studies characterize the physico-chemical properties of the silver nanoparticles used in each study summarized herein.

Oral Studies

There are currently three studies in the scientific literature that investigate the oral toxicity of nanosilver in rats and two studies completed with mice. The first rat study reported findings after

28 days of repeated gavage administration of carboxymethyl cellulose (CMC)-coated nanosilver with average diameter of 60 nm (minimum diameter of 53 nm and maximum diameter of 71 nm) to four-week old male and female Sprague-Dawley rats (n = 10 per dose) (Kim *et al*., 2008). There were liver effects (dilation of the central vein, bile-duct hyperplasia and increased foci), a coagulative effect on peripheral blood, and an increase in serum alkaline phosphatase (ALP) and cholesterol. There was a dose-dependent increase in silver distribution in many tissues (liver, kidneys, stomach, brain, lungs, testes, and blood) and a two-fold higher accumulation of silver in the kidneys of female rats when compared with male rats was also reported for all dose groups. A no-observed-adverse-effect level (NOAEL) of 30 mg/kg/day (lowest dose level) was reported based on the observed liver effects and increase in ALP and cholesterol at 300 mg/kg/day (mid-dose level).

The second rat study was performed by the same research group using four-week-old Fisher rats (n = 10 per dose) for 90 days (Kim *et al*., 2010). This study involved repeated gavage administration of CMC-coated nanosilver with average diameter of 56 nm (minimum diameter of 25 nm and maximum diameter of 125 nm) to rats and reported similar findings as the 28-day study including gender-related distribution of silver in the kidneys and a reported NOAEL of 30 mg/kg/day. However, intestinal pigmentation from exposure to nanosilver was reported, which was not observed in the 28-day study (Kim *et al*. 2008).

The third rat study involved feeding nanosilver (14 nm average diameter) stabilized with polyvinylpyrrolidone (PVP) or silver acetate to four-week old male (n = 6 per dose) and female (n = 10 per dose) Wistar Hannover Galas rats for 28 days (Hadrup *et al*., 2012a). They also reported no observed effects on the microbiological status of the rat's gastrointestinal tract following ingestion of nanosilver. However, Hadrup *et al*. (2012a) did report effects for silver acetate at a dose of 14 mg/kg/day including an increase in ALP, decrease in plasma urea, and lower thymus weights. Hadrup *et al*. (2012b) compared the neurotoxic effects of PVP-stabilized nanosilver (average diameter of 14 nm) and silver acetate, both, *in vivo* and *in vitro*. Following 28 days of oral administration, nanosilver (4.5 and 9 mg/kg/day) and silver acetate (9 mg/kg/day) significantly increased the concentration of dopamine in the brains of Wistar female rats, while the brain concentration of 5-hydroxytryptamine (5-HT) was increased only by nanosilver at a dose of 9 mg/kg/day. The NOAEL for this study was 9 mg/kg/day, but as this was the highest dose tested, a LOAEL could not be established. However, in the 14-day range-finding component of the studies, the brain dopamine concentration decreased in rats treated with nanosilver at doses of 2.25 and 4.5 mg/kg/day. In the *in vitro* experiment, three solutions consisting of 1) nanosilver, 2) an ionic silver solution obtained by filtering a nanosilver suspension, and 3) silver acetate were tested in neuronal-like PC12 cells. Nanosilver did not induce necrosis; however, cell viability was decreased and apoptosis (involving both the mitochondrial and the death receptor pathways) was found with all three solutions with the silver acetate being most potent.

There is one mouse study of repeated administration of nanosilver with average diameter of 42 nm (minimum diameter of 25 nm and maximum diameter of 55 nm) to mice for 28 days (Park *et al*., 2010). The study reported that, after oral administration of nanosilver at the dose levels of 0.25 mg/kg/day, 0.5 mg/kg/day, or 1.0 mg/kg/day, the serum enzyme levels of ALP and aspartate transaminase (AST) were significantly elevated in both male and female mice in the high dose group. The level of alanine transaminase (ALT) was also elevated in the high dose females. Histopathological analysis was performed in the high-dose groups and revealed that tissue change (*i.e*., slight cell infiltration) was observed in the cortex of the kidneys in both male and female mice, but no other histopathological changes were found in the portions of liver or small intestines that were examined. A NOAEL of 0.5 mg/kg/day was initially reported, based on the observed findings of elevated ALP, AST, and ALT and the histopathological changes in the kidneys at the 1.0 mg/kg/day dose level. However, for the 2015 Decision Document, EPA concluded that although the results of the Park *et al*. (2010) study showed evidence of changes in clinical chemistry, it lacked histological support for the effects used as the basis for the study NOAEL/LOAELs. In the absence of histopathological findings, the clinical chemistry changes observed alone are insufficient evidence of an adverse effect. The NOAEL is 1.0 mg/kg/day, the highest dose tested.

Liu *et al*. (2013) reported that nanosilver did not affect spatial cognition or hippocampal neurogenesis in adult male ICR mice (n = 15 per dose, n = 10 for control). Adult mice were administered nanosilver with average diameter of 51.4 nm by Dynamic Light Scattering (DLS) and 26.3 nm (size range from 10 to 70 nm) by Transmission Electron Microscopy (TEM) via intraperitoneal injection, at doses of 0, 10, 25, or 50 mg/kg, once a day in the morning for 7 consecutive days. Another group of mice received scopolamine (3 mg/kg) as a positive control for the behavioral studies. Investigators used the Morris water maze (MWM) test to evaluate spatial cognition and bromodeoxyuridine for detecting proliferating cells to measure neurogenesis. The test results showed that both reference memory and working memory were not impaired in nanosilver exposed groups compared with the control group, and no differences were revealed in hippocampal progenitor proliferation, new born cell survival, or differentiation in nanosilver treatment groups, indicating that neurogenesis was also unaffected.

Inhalation Toxicity Studies

The 2015 Registration Decision for NSPW-L30SS included a discussion of subchronic inhalation toxicity of nanosilvers in the public literature. These studies are not considered to be pertinent for the current assessment based on the lack of potential inhalation exposures to NSPW-L30SS.

Dermal Toxicity Studies

There are two dermal toxicity studies available for nanosilver. One study was performed to determine liver, skin and spleen pathologies of five- to six-week-old male guinea pigs (n = 6)

after exposure to nanosilver (concentrations 100 µg/mL, 1000 µg/mL, and 10,000 µg/mL) with a particle size of less than 100 nm based on exposure to aqueous solution using $AgNO_3$ as a positive control (Korani *et al*., 2011). The nanosilver suspension was applied by daily rubbing to an area of 5 cm by 5 cm (10% surface area) on the back of the animal with no wipe off or removal of chemical mentioned. The applied dose in mg/kg was not determined in the study. During the 13-week study, dose dependent and nanosilver specific effects were seen in the liver, the spleen and on the skin. Skin effects included decreased thickness of the epidermis and dermis, inflammation, increased levels of round cells, acidophilic cytoplasm in muscle fibers and increased levels of macrophages in the endomysium.

Liver effects included overproduction of Kupffer cells and degeneration of hepatocytes in a dose dependent fashion and necrosis at the 10,000 µg/mL concentration. Other dose-dependent effects observed in the liver included the following: thinner red capsules, inflammation, accumulation of red blood cells, and white pulp atrophy along with hepatic cord destruction. It does not appear that gauze or another dressing was used to cover the dosed area. No hematology, clinical chemistry or urinalysis was performed. Only the liver, spleen and skin were examined histopathologically.

Another study was performed to determine the effect of skin exposure to nanosilver (size = 20 and 50 nm, concentration = 0.34, 3.4 and 34 µg/mL aqueous solution) in 2 female pigs (Samberg *et al.*, 2010). A 500 µL nanosilver suspension was placed on one of 14 spots on the back of a hair-clipped pig, allowed to air dry, then covered with a Hilltop chamber (occlusion pad) and secured with non-irritating tape. This was followed by a body stocking covering the dorsum of each pig. Actual exposure was 0.6, 6, and 60 ng/mm^2 and total exposure was 0.17, 1.7 and 17 µg per dosing period. No gross pathological effects were noticed on the porcine skin. A concentration, but not particle size or washing state, dependent effect was seen on the dermal layers under microscopic investigation. Low dose effects were slight intra- and intercellular epidermal edema. Intermediate effects were a more focal intra- and intercellular epidermal edema alongside focal dermal and epidermal inflammation. The highest dose caused severe edema, with severe focal dermal inflammation, epidermal hyperplasia and parakeratosis. Precautions were taken to prevent oral dosing, including restraint/anesthesia and multiple covering layers. Only effects relating to the skin were examined.

None of the above studies are an acceptable substitute for a dermal subchronic study or a dermal irritation study. In no case was a full gross or microscopic histopathology panel performed, even in the cases where dose dependent effects of nano-silver particles were seen. None of these studies used the same particle size, coating type, or washing state, making it difficult to generalize information from them onto other products. The Korani *et al*. (2011) study lacked any clinical chemistry, hematology, or urinalysis of the guinea pigs. This study also could not draw NOAEL/LOAEL conclusions given that effects were seen at all levels of dosing. In the Samberg

et al. (2010) study, no systemic histopathological evaluations were possible, as multiple dose concentrations were tested on the same animal.

However, there is one published report on nanosilver concerning nanosilver used in wound dressings for patients suffering from burns. In this report, a burn patient using a nanosilver-coated wound dressing developed clinical signs of argyria and elevated serum liver enzymes indicative of liver toxicity along with elevated silver concentrations in blood and urine (Trop, 2006). This study indicates to EPA that nanosilver can be systemically absorbed when a large area of the skin barrier is severely compromised.

In the absence of acceptable dermal toxicity studies, EPA uses route to route extrapolation. However, use of the oral endpoint for evaluating dermal toxicity requires knowledge regarding the amount absorbed through the skin.
Currently, there is no guideline or scientific literature study conducted in animals for the *in vivo* dermal absorption of the nanosilver in NSPW-L30SS or of nanosilver available to EPA. However, there is a human clinical study, which is observational, that examined silver levels in serum and urine after application of burn wound cream containing silver sulfadiazine nanosilver (Wan *et al*., 1991). EPA used this information to derive a conservative DAF of 6.7% for nanosilver. A study completed by Brandt *et al*. (2012) demonstrated that nanosilver and silver sulfadiazine have similar skin absorption characteristics in mice after normalizing for silver dose. In addition, an *in vitro* study with nanosilver in human skin is available in the scientific literature. This study demonstrated that nanosilver penetration is very low for both intact and abraded skin at 0.00066% and 0.0033%, respectively (Larese *et al*., 2009).

Evidence of Neurotoxicity
There were potential neurotoxic effects identified with increases in neurotransmitter concentrations and decrease in spatial cognitive ability; however, these same effects were not observed in a follow-up study.

The study completed by Hadrup *et al*. (2012b), which reported significant increases in neurotransmitter concentrations (*e.g*., dopamine) after oral administration of nanosilver to rats at concentrations of up to 9 mg/kg/day, lacks histological support for determining NOAEL/LOAELs. The study by Liu *et al*. (2013) reported no effects on spatial cognition or hippocampal neurogenesis of mice after injecting nanosilver into the body cavity (*i.e*., intraperitoneal injection) at concentrations of up to 50 mg/kg. Therefore, EPA believes that the short-term incidental oral NOAEL and intermediate-term LOAEL of 30 mg/kg/day are expected to be protective of neurotoxic effects of nanosilver.

The effects on spatial cognition and hippocampal synaptic plasticity observed by Liu *et al*. (2012) after administering nasal drops containing nanosilver at concentrations of 3 and 30 mg/kg to rats suggest possible neurotoxic effects from the inhalation of nanosilver. Based

on the formulation of the product into a master batch, no inhalation exposures are expected from this product.

Reproductive and Developmental Toxicity Studies

There are studies showing dose-dependent increases in the concentration of silver in the testes of rats after oral ingestion, inhalation, and injection of nanosilver (see Section 3.4); however, there are few studies available on the reproductive and developmental toxicity of nanosilver.

There is an *in vitro* study investigating the toxicity of 15 nm nanosilver on spermatogonia isolated from 6-day-old mouse testes and immortalized with SV40 large T antigen (Braydich-Stolle *et al*., 2005). In this transformed (*i.e*., immortalized) cell line, both nanosilver and silver ions individually caused altered cellular morphology, decreased mitochondrial activity (as indicated by MTS cell viability assay), and increased apoptosis at doses up to 10 µg/mL; however, the effects from nanosilver were greater than observed for silver ions.

In another study, Austin *et al*. (2012) investigated the distribution of citrate-coated nanosilver with diameters between 30 and 60 nm and silver nitrate in pregnant mice (n = 6 to 12 per dose) and developing embryos. Nanosilver suspensions and silver nitrate were administered by intravenous injection (i.v.) on gestation days (GD) 7, 8, and 9 at nanosilver concentrations of 0, 0.4, and 0.73 mg/kg/day. Austin *et al*. (2012) reported a significant increase in nanosilver content as compared to silver nitrate treated animals in nearly all tissues; nanosilver accumulation was significantly higher in liver, spleen, lung, tail (injection site), visceral yolk sac, placenta, and endometrium. This study did not find significantly higher nanosilver in the ovary. Nanosilver was identified in vesicles in endodermal cells of the visceral yolk sac. This study demonstrated that nanosilver distributed to major maternal organs and extra-embryonic tissues, but the authors stated that very little silver reached developing embryos and no adverse morphological effects on the developing embryos were observed.

In a 14-day repeated maternal dose study (Yu *et al*., 2013), nanosilver (7.5 nm) was administered via gavage to 11 pregnant Sprague-Dawley rats per dose from days 6 to 19 of gestation. No gross pathological effects or other developmental effects on the fetus were observed up to the maximum dose of 1,000 mg/kg/day. There were no effects on maternal food consumption or body weight, but there were some slight changes in liver catalase and glutathione reductase levels (all dose levels) and a decrease in glutathione at the maximum dose. No effects were seen in any organs, including the ovaries. The teratogenicity potential of nanosilver in pregnant rats was investigated by Mahabady *et al*. (2012). Nanosilver of unknown size and surface coating was administered to pregnant rats via intraperitoneal injection (i.p.) on GD 8 and 9. Fetuses collected on GD 20 from animals that received nanosilver were reported to have reduced weight and length, but there were no effects on the skeletal system as compared to animals treated with a saline control. Because Mahabady *et al*. (2012) did not report key information about the

nanosilver particle size and surface coatings, it is not possible to compare these results to the nanosilver in any other study or in any nanosilver-containing product.

Mutagenicity Studies

There are no studies in the scientific literature that investigate the potential of nanosilver to cause cancer (*i.e*., carcinogenicity). The exposures from this chemical are not expected to be long term. Although no study investigating carcinogenicity is needed, a summary of studies follows relating to the potential of nanosilver to induce changes in genetic material (*i.e*., mutagenicity, genotoxicity). The *in vitro* results are equivocal as to the cytotoxicity and genotoxicity of nanosilver, using traditional mutagenicity tests including the bacterial reverse mutation assay (*i.e*., Ames test), the mouse lymphoma forward mutation test, the mammalian cell chromosome aberration test in Chinese hamster ovary cells, and the mouse lymphoma Comet assay for oxidative damage. Additionally, nanosilver was investigated in a series of *in vivo* studies. Therefore, there is no concern for mutagenicity or genotoxicity. Genotoxicity or mutagenesis is a key initiating factor for induction of carcinogenesis as changes in the DNA sequence can lead to the production of mutant proteins that alter the ability of the cell to maintain its normal cell division pathway If the checkpoints that maintain the cell from unchecked cellular division are altered, the cell go undergo proliferation that can then lead to outgrowth and thus, tumor formation (carcinogenesis). Results from these assays are summarized below.

The results of the studies summarized below are sufficient for risk assessment. The Agency is not requesting additional mutagenicity study because new data will not provide information that would change the conclusions on mutagenicity of nanosilver or NSPW-L30SS. Furthermore, since there is confounding data on the mutagenicity data from open literature studies on nanosilver, the Agency has determined that requesting more mutagenesis studies would not provide definitive data as to the mutagenic potential of nanosilver

In vitro reverse gene mutation assay in bacterial cells: The mutagenicity of nanosilver (average diameter of 10 nm) suspended in a 1% citric acid solution was determined at concentrations of up to 500 µg/plate in bacterial cells using the Ames test (Kim *et al*., 2012). Although cytotoxicity was observed at 31.25 µg/plate, nanosilver did not induce a mutagenic effect in the histidine-requiring strains of *Salmonella typhimurium* TA98, TA100, TA1538 and TA1537 or in tryptophan-requiring *Escherichia coli* strain WP2uvrA with or without the metabolic activation system (±S9).

A similar result was obtained by Li *et al*. (2012) for nanosilver with an average diameter of 5 nm (size range from 4 to 12 nm) prepared in TEM for the primary particles and for particles with a diameter of 1,608.7 ± 175.4 nm prepared in culture media. In this test, there was no increase in revertant mutant colonies of the standard *S. typhimurium* tester strains, which included the strain used to detect oxidative damage (TA102) up to cytotoxic concentration (31.25-62.5 µg/plate -S9; 125-250 µg/plate +S9). However, Li *et al*. (2012) cautioned that because of cytotoxicity and the

physical properties of nanosilver, this test system may lack the sensitivity required to detect the mutagenic action of the test material.

In vitro forward gene mutation assay in mouse lymphoma cells with a Comet Assay: The mutagenicity of nanosilver, which had an average diameter of 5 nm (size range from 4 to 12 nm) prepared in TEM for the primary particles and a diameter of 1,608.7 ± 175.4 nm in the culture media, was evaluated in mouse lymphoma L5178Y at the $TK^{+/-}$ locus and the modes of action was assessed using standard alkaline and enzyme-modified Comet assays with a gene expression analysis (Mei *et al*., 2012). Nanosilver induced dose-dependent cytotoxicity and mutagenicity with a marked increase in the mutation frequency at 4 and 5 µg/mL (<50% cell survival at ≥5 µg/mL) where nanosilver had a clastogenic mode of action. Subsequent testing revealed no evidence of DNA damage (Comet test) but oxidative damage (modified Comet test), confirmed by gene expression analysis, which showed an expression pattern consistent with production of reactive oxygen species (ROS).

In Vitro Chromosome Aberration Test: The clastogenic effect of nanosilver (average diameter of 10 nm) suspended in a 1% citric acid at concentrations of less than 31.25 µg/mL was determined in Chinese hamster ovary cells (CHO-k1) after 6- and 24-hour exposures ±S9 using OECD Test Guideline 473 (Kim *et al*., 2012). Nanosilver did not induce any statistically significant increase in the number of cells with chromosome aberrations, polyploidy, or endoreduplication when compared with the control group at concentrations causing approximately 50% cytotoxicity.

In Vitro Mammalian Cell Assays: An *in vitro* mammalian cell micronucleus test (OECD Test Guideline 487) was used to determine the genotoxicity of nanosilver, which had an average diameter of 5 nm (size range from 4 to 12 nm) by TEM for the primary particles and a diameter of 1,608.7 ± 175.4 nm prepared in culture media. Nanosilver at concentrations of up to 30 µg/mL induced a significant and dose-related increase in micronuclei in human lymphoblastoid TK6 cells, indicating that nanosilver has weakly positive genotoxic potential (Li *et al*., 2012).

The genotoxic effects of silver nanoparticles (AgNPs) (43-260 nm) were further studied by Kim *et al*. (2011) on normal human bronchial epithelial (BEAS-2B) cells using the *in vitro* micronucleus and Comet assays. As expected, ≈100% of the particles with diameters in the range of 40-55 nm were taken up by the cells; ≥50% of the particles in the range of 60 to 100 nm were found within the cells, and lower percents of the larger size particles were also deposited in the cells. Significant and concentration-related increases in DNA damage and micronuclei induction were found in the bronchial cells exposed to concentrations of 0.01 to 10 µg/mL silver nanoparticles. Significant and dose-related intracellular generation of reactive oxygen species (ROS) was also demonstrated at 0.01 to 10 µg/mL AgNPs. Finally, the investigators present data indicating that the DNA damage induced by AgNPs in BEAS-2B cells was blocked by several well-known ROS scavengers, primarily superoxide dismutase (SOD).

In Vivo Studies: In contrast to the above *in vitro* findings, the oral gavage administration of CMC-coated nanosilver with average diameter of 60 nm (minimum diameter of 53 nm and maximum diameter of 71 nm) caused overt toxicity but failed to induce an increase in micronucleated polychromatic erythrocytes (MN PCEs) in the bone marrow of male and female rats after 28 days of treatment at doses 0, 30, 300, and 1,000 mg/kg/day (Kim *et al*., 2008). This study indicates that nanosilver is neither clastogenic nor aneugenic *in vivo*, although a limitation of this study is that no measurements were performed to determine if nanosilver reached the bone marrow. Nevertheless, subsequent studies discussed below provide confirmation that silver nanoparticles are readily absorbed and distributed throughout the rodent body.

Negative results were also obtained by other researchers (Kim *et al*., 2011) using the inhalation route for a rat micronucleus assay.

In a series of *in vivo* studies, Li *et al*. (2013) found that various types of AgNPs ranging in size from 15-100 nm or 10-80 nm (coated with either PVP or silicon, respectively) were neither clastogenic, aneugenic, nor mutagenic in the mouse bone marrow micronucleus or *Pig-a gene* mutation assays. Tissue distribution was confirmed at the highest dose tested (25 mg/kg), following intravenous injection once of 5 nm PVP-coated AgNPs or daily for 3 days of 15-100 nm PVP- or 10-80 nm silicon-coated AgNPs. At 25 mg/kg, PVP-coated AgNPs caused significant ≈30% reductions in the percentage of reticulocytes. By contrast, no cytotoxicity occurred after treatment with 10-80 nm silicon-coated Ag particles. Thus, there is compelling evidence that PVP-coated AgNPs reached the target tissue but did not induce a mutagenic response in mouse bone marrow cells. Similar results indicate that PVP- or silicon-coated 25 mg/kg silver nanoparticles reached the target organ but were not genotoxic. However, both PVP- and silicon-coated 25 mg/kg silver nanoparticles induced significant oxidative damage in the mouse liver, manifested as binding to 8-oxoguanine adducts in the modified Comet assay.

In another *in vivo* test, Cho *et al.,* (2013) exposed male rats to concentrations of silver nanoparticles (14-15 nm) ranging from 0.66 x 10^6 particles/cm^3 (≈49 µg/m^3) to 3.24 x 106 particles/cm^3 (≈381 µg/m^3) 6 hours/day for 12 weeks. Lung cells were harvested, and DNA damage was assessed using the Comet assay. No significant changes in body weight or lung weight were observed. Histopathological examination of the lungs revealed an increased incidence of perivascular and chronic inflammation, accompanied by alveolitis, granulomatous lesions and alveolar wall thickening and macrophage accumulation. This inflammatory response paralleled a significant and dose-dependent increase in silver nanoparticles and DNA damage in lung tissue. The genotoxic effect at the high dose was significant and ≈2X higher than effects seen in the control.

In summary, the data from the *in vitro* studies (*i.e.*, Ames bacterial gene mutation, mammalian cell mutation and chromosome aberration assays) indicate that nanosilver is not expected to be mutagenic in bacteria. In mammalian cells, however, the *in vitro* micronucleus test and the mouse lymphoma Comet assay suggest that nanosilver may have mutagenic potential. This is consistent with a recent review article which stated that *in vitro* data suggest that nanosilver harbors mutagenic properties (Bartłomiejczyk *et al*., 2013). However, the lack of mutagenicity in several well-conducted *in vivo* studies, showing cytotoxicity and evidence that the nanoparticles reached the target tissue, suggests that the *in vitro* mutagenic potential observed in some of the *in vitro* studies may be intrinsic to nanoparticles but is not expressed in whole animals. There is evidence that silver nanoparticles are genotoxic, expressing DNA damage in cultured human lung cells and in the lungs and liver of intact animals (Comet assay) via intracellular generation of ROS. These findings are supported by the inflammatory processes described above in lungs. However, since initiation of ROS-induced oxidative DNA damage can be blocked by ROS scavengers, there is not a concern for mutagenicity or genotoxicity.

Silver Ion Toxicity

Humans may also be exposed to silver ions that would be released by NSPW-L30SS. Conventional silver, and the silver ions it releases, can qualify as pesticides under FIFRA when used for pesticidal purposes. The 2009 SAP concluded that the hazards of silver ions would be the same, whether they came from conventional silver or from silver nanoparticles. With respect to silver ions, EPA evaluated the toxicity and exposure to silver ions and determined that unreasonable adverse effects from use of silver-containing products are unlikely (U.S. EPA, 1993).

Absorption, Distribution, Metabolism, & Elimination (ADME)

Studies were completed using either injection or oral administration of nanosilver to laboratory animals to determine the absorption, distribution, metabolism, and excretion (ADME) of nanosilver in whole animals. Based on the studies described in the following sections EPA believes biliary or fecal excretion of nanosilver is the primary elimination pathway. Silver is found primarily in the liver, spleen, and kidneys, but also in the thymus, brain, heart, lungs and testes of animals dosed with nanosilver, silver nitrate, and silver acetate, where the organs of animals dosed with silver nitrate and silver acetate contained greater amounts of silver than did animals dosed with nanosilver. Animals can clear silver from blood and most organs given enough time but retain silver in the testes and brain. Animals treated with silver nitrate, silver acetate, and nanosilver all contain silver granules with dimensions on the nanoscale. However, it is unclear if intact nanosilver is absorbed into tissues or if nanosilver dissolves into ionic silver before being absorbed into tissues and forming nanoscale granules.

<u>Injection of Nanosilver</u>

The translocation, distribution and accumulation of silver after a single subcutaneous injection at 62.8 mg/kg of body weight of nanosilver with diameters between 50 and 100 nm and microsilver with diameters between 2,000 and 20,000 nm was determined in Wistar female rats (n = 30 per group) (Tang *et al*., 2009). The silver content of feces between 2 and 24 weeks after injection was significantly higher than in urine for both the nanosilver- and microsilver-injected animals, which suggests that both nanosilver and microsilver are eliminated through biliary excretion. Although there was no significant difference between the amount of silver at the injection site or in excrements after administration of nanosilver or microsilver, the amount of silver in organs was significantly greater for nanosilver. Animals injected with nanosilver were found to contain significantly more silver in the liver, kidney, spleen, brain, lung and blood than in animals injected with microsilver. Histopathological observations found that nanosilver-injected animals contained elemental silver spheres that were absent from the microsilver-treated animals. The elemental silver spheres were observed in different kinds of cells, such as renal tubular epithelial cells and hepatic cells. Moreover, these elemental silver spheres also induced blood-brain barrier (BBB) destruction and astrocyte swelling and caused neuronal degeneration.

The serum kinetics, tissue distribution, and excretion of silver after single injections of 0.5 mg/kg and 5 mg/kg of citrate-coated nanosilver with average diameter of 7.9 ± 0.95 nm was determined in SPF New Zealand White rabbits (n = 4) (Lee *et al*., 2012). There was no significant general toxicity reported in either the 0.5 or 5 mg/kg treatment groups. Accumulation of silver was observed in all the tested organs including liver, kidney, spleen, lung, brain, testes, and thymus, where the liver and spleen contained the greatest amount of silver. As with the rat study above, the amount of silver in feces between 1 and 28 days after injection was significantly greater than in urine, which suggests biliary excretion of silver is the major route of elimination after injection of nanosilver.

Blood kinetics, tissue distribution, and organ accumulation of silver were determined after daily intravenous injections for 5 consecutive days of between 23.8 and 27.6 mg/L of nanosilver with average diameters of 20, 80, and 110 nm in six-week-old male Wistar rats (n = 21 in treatment groups; n = 2 in control) (Lankveld *et al*., 2010). The concentration of silver in blood rapidly decreased during the initial 10 minutes following both single and repeated injection of nanosilver and then remained stable for up to one hour after injection. Silver was distributed to all organs evaluated, including the liver, lungs, spleen, brain, heart, kidneys, and testes, regardless of the size of nanosilver injected. After injecting 20-nm diameter nanosilver, silver was found to be distributed mainly to the liver, followed by the kidneys and spleen, whereas after injection of the 80- and 110-nm diameter nanosilver, silver was distributed mainly to the spleen, followed by the liver and lung. Thus, there was a size-dependent tissue distribution. Repeated administration of nanosilver resulted in accumulation of silver in the liver, lung, and spleen, indicating that these organs may be potential target organs for toxicity after repeated exposure.

Oral Administration of Nanosilver

The organ distribution and cellular localization of silver was determined following 28-day repeated oral administration at 9.0 mg/kg/day of PVP-stabilized nanosilver with average diameter of 14 ± 4 nm and silver acetate to four-week-old female Wistar Hannover Galas rats (n = 9 for nanosilver and n = 7 for silver acetate) (Loeschner *et al*., 2011). Although the distribution of silver in organs for animals treated with nanosilver and silver acetate was similar, the concentration of silver in the organs treated with silver acetate was greater than it was for nanosilver-treated animals. This was in agreement with the higher fecal excretion of nanosilver as compared to silver acetate. Besides the intestinal system, the largest silver concentrations were detected in the liver and kidneys; however, silver was also found in the lungs and brain. Remarkably, silver-containing granules in the same size range as that of the administered nanosilver were observed in the ileum and kidney tissues of rats exposed to nanosilver and silver acetate. Using transmission electron microscopy (TEM), sulfur- and selenium-containing granules were detected in the ileum of animals exposed to nanosilver and silver acetate and were mainly located in the basal lamina of the ileal epithelium and in lysosomes of macrophages within the lamina propria. The results of the present study demonstrate that the organ distribution and form of silver were similar when nanosilver or silver acetate were administered orally to rats.

The toxicokinetics and tissue distribution of silver was determined following 28-day oral gavage at 90 mg/kg/day of nanosilver with average diameter of 17.7 ± 3.3 nm by TEM, PVP-coated nanosilver with average diameter of 12.1 ± 8.0 nm by TEM, and 9 mg/kg/day of silver nitrate to six-week-old male pathogen free Sprague-Dawley rats (n = 5 per group) (Van der Zande *et al*., 2012). Greater than 99% of the silver administered to the rats was excreted in their feces indicating that only a small fraction (<1%) of silver from nanosilver and silver nitrate was absorbed. After normalizing for gavage dose, the concentration of silver in the blood of the silver nitrate treated animals was significantly higher than for the nanosilver-treated animals at all timepoints during exposure. This clearly illustrates a much higher uptake of silver when silver nitrate was administered as compared to nanosilver. One day after the oral gavage, a significant reduction in blood silver concentration was observed. One week after the oral gavage, the concentration of silver in blood was reduced to nondetectable levels indicating rapid clearance of silver from the blood for both nanosilver and silver nitrate treated animals. After normalizing for gavage dose, silver was observed in all examined organs with the highest levels in the liver and spleen where animals treated with silver nitrate had accumulated significantly more silver than in animals treated with nanosilver. Silver was cleared from most organs eight weeks after the final gavage, but remarkably not from the brain and testes, where between 94 and 100% of the silver was still present in the brain compared to the amount one day after the last gavage of nanosilver and silver nitrate, respectively. Using single-particle inductively coupled plasma mass spectrometry, nanosilver was detected one day after the final gavage in the liver, spleen, lungs, and gastrointestinal contents of nanosilver gavaged rats. Nanosilver was also detected in the

liver, spleen, lungs, and gastrointestinal contents one day after the final gavage from silver nitrate treated rats demonstrating the in vivo formation of nanosilver from silver nitrate. Blood enzyme levels were not significantly different from untreated animals, indicating that there was no acute hepatotoxicity observed. Also, there was no indication that nanosilver caused nonspecific immune responses based on immunotoxic responses.